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A study of the influence of antimicrobial agents on the aerobic flora of the bowel

E.J. Vollaard

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van de geneeskunde en de tandheelkunde

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Ernst Joan Vollaard
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Promotor : Prof. dr. J.W.M. van der Meer

Co-promotor : Dr. H.A.L. Clasener

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CHAPTER I

INTRODUCTION

In 1979 a symposium was organized in Utrecht, called "New Criteria for Antimicrobial Chemotherapy : Maintenance of Digestive Tract Colonization Resistance" (1). In this symposium a strong plea was made to give preference to antimicrobial agents that do not disturb the anaerobic flora that provides colonization resistance (CR) against potentially pathogenic aerobic flora. In this way the risk of provoking superinfections by antimicrobial therapy would be eliminated. Moreover, administration of antimicrobial agents that attain high concentrations in the digestive tract while sparing the anaerobic flora that provides CR, would offer a method of long-term antimicrobial prophylaxis in high risk patients. This method was called "selective decontamination". Successful application of selective decontamination in neutropenic patients had been reported since 1977 (2), but (surprisingly) the method was not applied in other groups of patients at high risk of infections.

At that time we did already depend heavily on co-trimoxazole and doxycycline in our hospital, not because they would spare CR, but because they were cheap and rather effective. In case of serious infections with Gram-negative bacilli we used cefotaxime, but in the lowest dosage calculated to be effective (3). So, in general our antibiotic policy was in agreement with the recommendations given by van der Waaij (1). The major difference was that there was no active policy to restrict the use of penicillins and clindamycin.

In 1983, at a symposium in Brussels, successful application of selective decontamination in mechanically ventilated patients was reported (4). At about that time dr. H. Clasener, who had worked with dr. R. van Saene in Groningen, started working in our hospital as a microbiologist and tried to convince everyone of the importance of the concept of CR as a guideline for antibiotic policy. We agreed that it would be justified to propose selective decontamination for neutropenic patients and for patients on mechanical ventilation in our hospital. However, because the Groningen-regimen was very expensive, we devised an alternative regimen, which was more than ten times cheaper (5). The favourable results we obtained with this alternative regimen in both kinds of patients (6, 7) strongly increased our conviction that the concept of CR was an important hypothesis for the selection of antimicrobial agents.

In the mean time we had started extensive study and lengthy discussions of available literature concerning this topic, resulting in

three review articles (8, 9, 10). The last of these is chapter II of this thesis. It summarizes our points of view in about 1988. Although our opinions have changed considerably since then, this review has been included in the thesis because it describes the historical background of the concept of CR.

Charmed by the concept of CR, we wanted to restrict as much as possible the use of antibiotics that disturb this mechanism of defense. However, the experiments on which the classification of antibiotics by van der Waaij depended, had been performed in mice only. The extrapolation of the doses used in mice to the comparable dosage in man seemed rather speculative. Especially the suggestion that some antibiotics would be "safe at any dosage" met our suspicion. Therefore, we decided to start this kind of investigations in man. In the first two studies (chapters III and IV) we used the conventional study design, which investigates the influence of antibiotics on the median concentration of aerobic flora in a group of individuals. We used volunteers in stead of patients, because CR may also be influenced by disease (chapter II). All volunteers were treated with one drug that was believed to impair CR (positive control), with one drug that was believed to spare CR (negative control), and with one or two drugs for which we (or the sponsoring company) wanted to establish the influence on CR. Although we presumed that microbial CR is provided exclusively by obligate anaerobic flora, we analysed aerobic flora only, because the composition of the anaerobic flora that provides CR is unknown and most anaerobic species cannot be cultured. Moreover, it cannot be excluded that CR can be impaired by a decrease of the production of inhibiting substances, despite unchanged concentration of the anaerobic flora that provides CR. Therefore, we decided to restrict ourselves to investigation of the impact of administration of antimicrobial agents on the concentration of aerobic flora of the digestive tract (which is the clinically important event anyhow).

Originally, we investigated microbial concentrations both in saliva and in faeces. However, because we did not observe an increase in the concentration of Gram-negative bacilli or yeasts in saliva following antimicrobial agents that did increase the concentration of those micro-organisms in faeces, only faeces was analyzed in subsequent investigations. Probably, demonstration of impairment of CR in volunteers is easier in faeces than in saliva, because in the

oropharynx other factors than anaerobic flora, for example the presence of fibronectin (see chapter II) or of alpha-haemolytic streptococci (11), are the dominant factors in CR against Gram-negative bacilli and yeasts.
The results of the first two studies were according to our expectations concerning the influence of the antimicrobial agents on the faecal concentration of Gram-negative bacilli and yeasts. Unexpectedly, low-level colonization by other Gram-negative bacilli than *Escherichia coli* (secondary colonization) appeared to be facilitated by agents like roxithromycin and doxycyclin, although the total faecal concentrations of Gram-negative bacilli, of enterococci and of yeasts did not demonstrate disturbance of CR following those agents. This posed the problem whether increase of secondary colonization is a more sensitive indicator of disturbance of the anaerobic flora that provides CR than the faecal concentration of aerobic flora, or that selective reduction of the concentration of *E. coli* enables low-level colonization by other species of Gram-negative bacilli ("substitution colonization").
After publication of those studies we realized ourselves new problems concerning the interpretation of the data. For example, following amoxycillin the concentration of Gram-negative bacilli and yeasts increased, but the concentration of enterococci decreased. The obvious explanation for this decrease, is that the volunteers did not harbour or acquire resistant enterococci. However, this implied that the decrease of Gram-negative bacilli observed following erythromycin, roxithromycin, co-trimoxazole, doxycycline and cefaclor could also be explained by absence of sufficiently resistant Gram-negative bacilli. Therefore, the absence of a spontaneous increase of the concentration of Gram-negative bacilli following those agents did not prove that they spare the anaerobic flora that provides CR. We concluded that the methodology should be improved by inclusion of a challenge with resistant micro-organisms. We hesitated to do so, however, because we heard that one of the volunteers of van Saene in Groningen had developed haemorrhagic colitis after ingestion of a *Klebsiella oxytoca* challenge strain during adminstration of amoxycillin.
A possible alternative for the use of challenge strains, was the faecal concentration of bèta-aspartylglycine, which increases when CR is impaired. However, this is a rather insensitive indicator of impair-

ment of CR, and we did not dispose of the required analytical equipment. Therefore we became interested in work of Midvedt, who studied the influence of antimicrobial agents on the concentration of several substances in faeces of laboratory animals. It appeared that the faecal concentration of urobilinogen decreased strongly following agents that (in our opinion) disturbed CR, and not following agents that did not. Therefore the faecal concentration of urobilinogen might be useful as a substitute for challenge with resistant micro-organisms in our investigations.

In chapter V we describe our study of the influence of four antibiotics on the faecal concentration of urobilinogen. The data confirmed the idea that major impairment of CR (following clindamycin or dicloxacillin), strongly decreases the faecal concentration of urobilinogen. However, though not statistically significant (P = 0.08), the concentration of urobilinogen also decreased following norfloxacin, which was considered not to impair CR. This might indicate that the urobilinogen-method was not sensitive enough to achieve statistical significance for a possible minor disturbance of CR following norfloxacin. In our view, this could only be investigated by returning to the original idea of using a resistant challenge strain. At that time we made regular surveillance cultures of faeces of an 85 year old diabetic patient in a nursing home, who was on continuous prophylaxis with norfloxacin 200 mg once daily for the prevention of urinary tract infections. Following 9 months of prophylaxis, this patient became colonized with a strain of *Klebsiella pneumoniae*, highly resistant to norfloxacin and amoxycillin, but very sensitive to co-trimoxazole and cefotaxime. Due to delays in communication, the patient was colonized for more than one month, in a concentration of about 10^8 cfu/gram faeces, before we started administration of colistin in order to eliminate this strain. Because high level colonization of the bowel in this old, diabetic patient did not cause her any problems, we considered it unlikely that this strain would cause problems in healthy volunteers. So we started administration of this strain to ourselves in increasing dosages, in order to study the rate of elimination from the bowel before and during administration of antibiotics. In this way we lost our fear for the use of a challenge with antibiotic-resistant micro-organisms. It can even be argued that adminstration of resistant challenge strains during treatment with antimicrobial agents is safer than eating food,

especially salads, because this contains Gram-negative bacilli with an unknown susceptibility profile.
The challenge strain was used in a study in which we compared the influence on CR of amoxycillin and of oral and parenteral pefloxacin. The results of these studies have been published in combination with new studies with amoxycillin (chapter VI), and pefloxacin (chapter X).
Another problem with the conventional methodology is that the data are analyzed for the whole group of volunteers. We realized that this is not logical, because the antibiotic susceptibility-profile of the anaerobic flora is not necessarily the same in all volunteers. Therefore, the influence of antimicrobial agents on CR may differ between volunteers. Actually, this might have been the explanation for our observation that some of the volunteers in the first amoxycillin-study did not show the expected increase in the faecal concentration of Gram-negative bacilli (chapter III). We concluded that we needed a method for statistical analysis of the data in each volunteer separately. This appeared to be a problem. The statisticians we approached stated that statistical analysis of data in individuals is impossible. A few months later however, one of the pharmacists in our hospital told me that he had discussed this issue with a statistician he knew, who assured him that statistical analysis on data of individuals is possible indeed, though it is rarely practised in medical studies. In this way dr. H. Wynne (from the Centre for Biostatistics, State University of Utrecht) made an essential contribution to the development of our study design, by making it possible to analyze the data in individual volunteers in subsequent studies.
The method of single person analysis enabled us to study the correlation between five different possible indicators of disturbance of CR in subsequent studies : The faecal concentration 1) of Gram-negative bacilli, 2) of aerobic Gram-positive cocci, 3) of yeasts, 4) of spontaneously acquired secondary Gram-negative bacilli (spontaneous challenge) and 5) of the challenge strain administered intentionally. As a working hypothesis, we supposed that impairment of the anaerobic flora that provides CR would cause an increase in indicators 1), 2) and 3), if resistant species are present or are acquired during the period of antibiotic-administration. Further, we expected that indicator 4) might be false-negative due to

the absence of resistant strains and false-positive due to "substitution colonization", and that indicator 5) would be the most reliable and most sensitive indicator of impairment of the anaerobic flora that provides CR.
The results of a new study with amoxycillin (chapter VI), demonstrated that the indicators 4) and 5) may also be false-negative due to competition between different species of Gram-negative bacilli. If for example amoxycillin disturbs CR in a volunteer, but his strain of *E. coli* is resistant to amoxycillin, this *E. coli* will grow out. If this occurs fast enough, *E. coli* may occupy all the increased "space" for Gram-negative bacilli that came available due to disturbance of CR, and may prevent secondary colonization by Gram-negative bacilli acquired spontaneously (indicator 4) or administered intentionally (indicator 5). The result of the challenge may also be negative because the challenge strain may loose the competition with secondary Gram-negative bacilli acquired before adminstration of the intentional challenge. Therefore, indicators 1) - 3) are more reliable indicators of the influence of an antimicrobial agent on CR than indicators 4) and 5), if Gram-negative bacilli resistant to that agent are prevalent. If such strains are rare however, as is the case with cefotaxime, facilitation of colonization by the challenge strain is a very reliable and possibly the most sensitive indicator of impairment of CR against bacteria (chapter VII).
As discussed before, the results in chapters III and IV suggested that it might be possible that selective elimination of *E. coli* (that is without impairing anaerobic flora) might allow low-level colonization by secondary Gram-negative bacilli ("substitution colonization"). In order to investigate whether this is true, resistant challenge strains should be administered after selective elimination of *E. coli*. However, because the anaerobic flora that provides CR is unknown, absence of impairment of this flora can not be proven. So, the only way to study the consequences of "selective" elimination of indigenous Gram-negative bacilli (mostly *E. coli*) is the administration of the lowest effective dosage of an agent that has a large difference between minimal inhibitory concentration (MIC) against Gram-negative bacilli on one hand, and against the most susceptible (known) species of anaerobic bacteria on the other hand. For this, quinolones are prime candidates. In a pilot trial we had found indications that the diffusible faecal concentration of

pefloxacin is about equal to the microbiologically active concentration in the bowel, and that a dosage of 800 mg daily results in an active faecal concentration of about 160 mg/gram (chapter XI). Therefore we decided to eliminate indigenous Gram-negative bacilli in our volunteers with a dosage of 20 mg pefloxacin daily, in order to achieve a diffusible faecal concentration of 4 mg pefloxacin/g faeces (chapter IX). This study showed that spontaneous absence of indigenous Gram-negative bacilli (as occurred in one volunteer), or elimination of *E. coli* and other indigenous Gram-negative bacilli from faeces, did not facilitate colonization of the bowel by a resistant challenge strain. Hence, if the phenomenon exists at all, substitution colonization does not occur easily. Therefore, it is our present view that increase of low-level secondary colonization is suggestive evidence for impairment of the anaerobic flora that provides CR.

Our studies confirmed the idea that impairment of the anaerobic CR-flora may cause a simultaneous increase in the faecal concentration of Gram-negative bacilli, aerobic Gram-positive cocci and yeasts (indicators 1-3), if resistant species are present. However, contrary to our hypothesis, this appeared not to be invariably so. In four of five volunteers with significant disturbance of the CR against aerobic Gram-positive cocci and yeasts following cefotaxime, the faecal concentration of a highly resistant challenge strain did not exceed the pretreatment concentration of indigenous Gram-negative bacilli (chapter VII), and one volunteer with significant disturbance of CR against Gram-negative bacilli and aerobic Gram-positive cocci following clindamycin, did not experience any increase of the faecal concentration of yeasts (chapter IX). At present, the explanation for these observations is unknown. It might indicate that there is some selectivity of anaerobic species concerning their inhibiting action on groups of aerobic flora. It is also possible that other factors than microbial CR played a role, for example secretion of antibodies or lack of important nutrients for certain micro-organisms in the bowel of some volunteers.

The study in chapter IX demonstrated that colonization of the bowel by *E. coli* or other Gram-negative bacilli, is not required for CR against yeasts or against streptococci and enterococci. So, "selective decolonization" of faeces from Gram-negative bacilli is possible. However, our original conviction that agents like cephradine, co-

trimoxazole, doxycycline, cefotaxime and quinolones do not impair CR is not right. The fact that these agents cause less overgrowth by resistant Gram-negative bacilli than amoxycillin is due to their superior activity against Gram-negative bacilli, and not to absence of impairment of anaerobic flora. These agents are unmasked, if the faecal concentrations of enterococci, yeasts or resistant challenge strains are analyzed.

Our studies indicate that most or all of the presently available antimicrobial agents disturb microbial CR when administered in standard dosage. So, the original purpose of our studies, *id est* discovering antibiotics that do not disturb CR, has not been achieved. However, we improved the study design for investigation of the influence of antimicrobial agents on CR. With this study design we could demonstrate that elimination of indigenous Gram-negative bacilli from faeces does not impair CR. Hence it is possible to use antimicrobial prophylaxis or therapy without increasing the risk of superinfections. Therefore the search for antimicrobial agents that do not impair CR should continue.

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CHAPTER II

EFFECT ON COLONIZATION RESISTANCE ; AN IMPORTANT CRITERION IN SELECTING ANTIBIOTICS

E.J. Vollaard[a], H.A.L. Clasener[b], H.K.F. van Saene[c] and N.F. Muller[a]

Departments of [a]Pharmacy and [b]Microbiology, Canisius-Wilhelmina Hospital, St. Annastraat 289, 6525 GT Nijmegen, The Netherlands
[c]Department of Medical Microbiology, University of Liverpool, England

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Abstract

Infections in humans are most often caused by aerobic micro-organisms colonizing the digestive tract. Aerobic micro-organisms are constantly entering the digestive tract with food, but colonization is resisted by autochthonous anaerobic flora (microbial colonization resistance) and by host-related factors (physiological colonization resistance). Antibiotics to which the autochthonous anaerobic flora is susceptible and that achieve sufficiently high concentrations at the sites of colonization will reduce colonization resistance (CR).
Consequently, resistant aerobic flora of the digestive tract may reach high concentrations, increasing the risk of superinfection. Therefore, when choosing antimicrobial agents for therapy, the effect on CR should be taken into account.
Immune-suppressed hosts and patients undergoing mechanical ventilation can be protected from serious infections by eliminating the most dangerous species of the aerobic indigenous flora, leaving CR intact. This is called selective decolonization. This article summarizes the effect of antimicrobial agents on CR

Introduction

Normal indigenous intestinal flora may be divided into autochthonous flora, consisting of anaerobes in high concentrations, and potentially pathogenic flora, consisting of aerobes in much lower concentrations. The concentration of potentially pathogenic aerobes is kept low by the anaerobic flora, annihilation of which may permit an enormous increase of potentially pathogenic micro-organisms in the intestine (1). The idea that the autochthonous anaerobic flora keeps the aerobic indigenous flora under control explains why antibiotics that disturb autochthonous anaerobic flora cause the occurrence of superinfections. This may also provide an explanation for the success of prophylaxis of endogenous infections in neutropenic patients by oral trimethoprim/sulfamethoxazole (not affecting anaerobes) although a combination of gentamicin and vancomycin (eliminating anaerobes) fails due to faecal overgrowth of resistant Gram-negative bacilli (1).
Concomitant with the growing understanding of why some anti-

biotics are not effective for long-term use, new antibiotics have been developed that do not lead to faecal overgrowth. The indication for long-term prophylaxis of endogenous infections might be extended from leukopenia to other populations (e.g., patients on mechanical ventilation), (2), and therefore it is useful to summarize available data.

Colonization of the digestive tract and pathogenicity of the normal flora

After birth, the skin and mucous membranes are colonized by large numbers of many different species of micro-organisms, which constitute the normal human flora (3). The gastrointestinal tract is extensively populated by aerobic and anaerobic bacteria. The oropharynx and the colon are colonized by the highest concentration of micro-organisms. Saliva contains about 10^9 anaerobic and 10^6 aerobic micro-organisms per millilitre. Faeces contain about 10^{11} aerobic and 10^7 aerobic micro-organisms per gram. The following review of normal gastrointestinal flora is necessary to understand the concept of CR.

anaerobes

Anaerobes of the gastrointestinal tract rarely produce endogenous infections, even when resistance to infection is markedly reduced. However, infection may occur in the event of pulmonary aspiration or intestinal surgery. The most common anaerobic species colonizing the colon (*Bacteroides* spp., 10^{11} organisms per gram of faeces) are also the most frequent causes of anaerobic infections following lower gastrointestinal surgery.
The colon harbours more than 400 different anaerobic species (4). Most of these are present in much lower concentrations than *Bacteroides*. Therefore they cannot be isolated by terminal dilution and special selective media are required. Moreover, anaerobic culture does not exclude most aerobic species, like *Escherichia coli* and enterococci, because these are facultative anaerobes. Because of these technical problems, knowledge of the anaerobic flora of the digestive tract is incomplete

Streptococcus viridans is always present in the oropharynx (10^7 organisms per millilitre of saliva), but rarely causes infections. Only in cases of underlying cardiac valvular disease or leukopenia is there a risk of endogenous infection. *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Branhamella catarrhalis* may be frequently found in throat cultures (5). If resistance is normal they are not pathogenic, but colonization may lead to infection when resistance is impaired (6). *Staphylococcus aureus* colonizes the nasal vestibule in many health persons. The nose is the primary source of colonization of both skin and intestinal staphylococci (3). *S. aureus* may be involved in early respiratory tract infections in patients with nasotracheal intubation for artificial ventilation (7).

E. coli (10^7 organisms per gram of faeces) is the predominant aerobic micro-organism of the lower gastrointestinal tract (8, 9) and the major pathogen of urinary tract infections (10). Normally *E. coli* is not found in the oropharynx (11). Other Enterobacteriaceae, such as *Proteus, Klebsiella, Enterobacter, Citrobacter*, and *Serratia* spp., as well as Pseudomonadaceae (e.g., *P. aeruginosa*) and *Acinetobacter* spp. (secondary aerobic flora) do not normally colonize the oropharynx (11) and colonize the intestine only to a small extent (8). These bacteria enter the gut in large numbers with food (12), but apparently do not displace *E. coli* as the predominant species in healthy subjects. With reduced resistance, colonization of the oropharynx (11), intestine (8) and vagina (13) occur, thereby increasing the risk of endogenous infections of the respiratory tract (14) and the urinary tract (13). Enterococcus colonizes the gut as frequently as *E. coli* but usually in lower concentrations. Enterococcus follows *E. coli* as the second most frequent cause of urinary tract infections in hospitalized patients (10).

Candida albicans occurs in low numbers in many healthy subjects in the oropharynx, intestine, and vagina. Local infection, like oral thrush or vaginitis, only occurs in patients with reduced antimicrobial defense. However, "persorption" (passage of viable microorganisms through intact gastrointestinal mucosa) has been described following ingestion of 10^{12} colony-forming units (cfu) of *C. albicans* in a healthy volunteer (16).

Invasive fungal infections may occur in compromised hosts (e.g.,

neutropenic or transplant patients) secondary to persorption of the gastrointestinal mucosa or other mechanisms of entry into the systemic circulation (17).

Colonization Resistance

Humans ingest millions of micro-organisms with food (12). Under normal circumstances these organisms do not persist in the digestive tract; however, when extremely large numbers of micro-organisms are ingested, they may be detected in the faeces for more than three days (1).

The ability to prevent or limit colonization of the digestive tract by micro-organisms is called CR (1). This resistance is mediated by numerous anatomic and physiologic factors, including intact mucosa, swallowing, salivation, secretion of immunoglobulin A and gastric acid, desquamation of cells of the mucous membranes, and normal gastrointestinal motility (18). These factors prevent adhesion of micro-organisms to mucous membranes and promote rapid gastrointestinal transit. CR may be impaired by illness (19), old age (20), hospitalization (8), and stress of surgery (21). Patients with these impairments more frequently have secondary flora in the oropharynx and intestine than do healthy young people.

The indigenous flora also plays an integral role in CR. In germfree mice and in mice that have been pre-treated with certain antimicrobials (e.g., streptomycin), the oral dose of exogenous micro-organisms that results in colonization is 1000- to 100,000 fold-less than that in untreated controls. Moreover, in mice that have been pre-treated with streptomycin and in germfree mice the concentration of aerobic Gram-negative challenge strains in faeces becomes 1000- to 100,000-fold higher than the normal level of *E. coli* (10^5 organisms per gram). This is followed by migration of viable *E. coli* from the gastrointestinal tract to mesenteric lymph nodes, the spleen, and other organs (1). Therefore, inhibition of autochthonous flora by antibiotics may decrease the dose of exogenous micro-organisms required to colonize the digestive tract and may increase the level of potentially pathogenic aerobic flora in the digestive tract.

Microbial CR has been ascribed to anaerobic flora alone (1), and to both anaerobic and aerobic autochthonous flora (22). In germfree

mice, oral administration of *Shigella flexneri* resulted in faecal concentrations of 10^9 organisms per gram. In germfree mice that had first been colonized with a cultured mixture of anaerobic bacteria, colonization by *S. flexneri* was limited to 10^5 organisms per gram of faeces. When *E. coli* was added to the cultured mixture of anaerobes, the faecal concentrations of *S. flexneri* was limited to 10^3 organisms per gram (24).

Based upon these studies, which are possible only in germfree animals, it appears that the anaerobic flora is the most important part of the CR flora, although *E. coli* may act synergistically with anaerobic flora in preventing low-level secondary colonization of the bowel with Enterobacteriaceae.

In order to impair CR, an antibiotic has to be not only effective against anaerobic bacteria but also present in sufficiently high concentrations in saliva, bile, intestinal mucus secretions, or faeces. This occurs when the antibiotic is incompletely absorbed or is sufficiently excreted in the digestive tract (25).

If the anaerobic flora is resistant to the agent in use, the antibiotic will not impair CR (26, 27). This might explain the interindividual differences observed in the influence of antibiotics on CR.

In humans also, the CR-flora appears to be mainly anaerobic, based on the following observations. Human anaerobic flora restores CR against *P. aeruginosa* in germfree mice (28). Cefoxitin increases the level of faecal Enterobacteriaceae, enterococci, and yeasts in human volunteers (29). The most plausible explanation for the increase of all three groups of aerobic micro-organisms following cefoxitin might be suppression of selected anaerobic flora. Quinolones, which have little impact on anaerobic flora, eliminate Enterobacteriaceae from the bowel and cause a small increase in the faecal concentration of yeasts in some volunteers (30). On the contrary, cefoperazone inhibits both anaerobic and aerobic species, resulting in a million-fold increase in the concentration of yeasts in the lower gastrointestinal tract (29). Clindamycin is effective against anaerobes, reaches high faecal concentrations, and strongly impairs CR (31).

Some antimicrobial agents that are effective against anaerobes (e.g. tinidazole and cephradine) do not reach the anaerobic flora of the gut in inhibitory concentration and therefore do not impair CR (1, 32). However, evidence that the CR-flora in humans is anaerobic will

remain circumstantial until the precise composition of this flora is known.

Summarizing, we propose the following concept of CR of the lower gastrointestinal tract. Normal physiology of the gut limits colonization by many micro-organisms. This physiological resistance does not prevent colonization of the colon by a large number of anaerobic species. The autochthonous anaerobic flora limits colonization of the gut by potentially pathogenic micro-organisms through an undetermined mechanism. We propose to call this mechanism anaerobic microbial CR. The combination of physiologic CR and anaerobic microbial CR does not fully prevent colonization of the bowel by *E. coli* and enterococci, yet it limits the concentration of these micro-organisms to about 10^7 cfu per gram of faeces. The combination of physiologic CR, anaerobic microbial CR, and the presence of indigenous aerobic bacteria in normal concentration may limit colonization of the bowel by other aerobic micro-organisms (secondary colonization), (9).

In the oropharynx also, the concentration of aerobic flora may be limited by anaerobic micro-organisms (33).

Superinfections

From the beginning of the antibiotic era, superinfections have represented a failure of treatment that initially seemed successful: adequate therapy may eliminate the infecting micro-organism, yet it is succeeded by a resistant micro-organism, usually of another species. In 1947 Weinstein proposed that superinfections following the use of antibiotics are caused by a disturbance of the autochthonous flora of the digestive tract (34). This insight did not have practical consequences until an animal model was developed by which the disturbance could be measured (1). Impairment of CR leads to "supercolonization" and subsequent superinfection: (1) New aerobic micro-organisms will now colonize the digestive tract, even when ingested in low numbers. (2) The aerobic flora (both primary and secondary) then reaches abnormally high concentrations. Therefore, superinfections will occur with considerable frequency after penicillin (especially at higher dosages), methicillin, tetracycline, chloramphenicol, and ampicillin. The species causing

these endogenous infections have frequently belonged to the Enterobacteriaceae, Pseudomonadaceae, or fungi (1).

An instructive example of the occurrence of superinfections was given by Price and Sleigh, who reported an epidemic with ampicillin-resistant *Klebsiella aerogenes* in a neurosurgical intensive care unit (ICU). Infections were noted 12 months after introduction of ampicillin and cloxacillin for prophylaxis and therapy. A marked increase in the number of patients with *Klebsiella* colonization was also observed. Modernization of the unit, sterilization of all equipment, and strict attention to nursing technique provided no improvement. After 11 patients had died of meningitis or pneumonia caused by this *Klebsiella*, it was decided to stop all antibiotics, both for therapeutic and for prophylactic indications. Soon after discontinuation of antibiotics, the *Klebsiella* infections resolved and the total number of infections within the ICU dropped markedly. The frequency of respiratory infections decreased from 45 to 15 percent and that urinary tract infections from 21 to 8 percent, leading to a threefold decrease in the rate of infection (35).

The occurrence of some epidemics with antibiotic-resistant strains in hospitals might be explained as follows. Widespread use of CR-impairing antibiotics such as ampicillin reduces CR of many patients. When these patients are contaminated with antibiotic-resistant microorganisms, bacteria may multiply to large numbers, increasing the risk of cross-colonization of other patients. In the presence of reduced resistance infection may ensue (27). The usual attempts to prevent cross-colonization consist mainly of hygienic measures (e.g., handwashing, isolation of infected patients, and elimination of environmental sources) and are generally unsuccessful (35). Better results are obtained by using not only proper hygiene, but also by avoiding those antibiotics that impair CR and by selective decolonization of high-risk patients (7, 36).

Occurrence of superinfection following antibiotic therapy is not confined to hospitalized patients. The use of oral amoxycillin and penicillin was regarded as the cause of an epidemic of resistant *Salmonella newport* infections. In this study, 12 of the 18 patients had taken amoxycillin or penicillin so shortly before the outbreak of salmonellosis that contamination of the antibiotics with *Salmonella* was initially suspected (37). It was subsequently thought, however, that the use of these antimicrobial agents greatly increased the

susceptibility to infection due to impairment of CR (38).
In a prospective double-blind study in a general practice population with respiratory infections, the effects of amoxycillin and cefaclor on colonization and occurrence of superinfections were compared. In the amoxycillin group (72 patients) colonization of the pharynx with potentially pathogenic micro-organisms occurred in 30 patients (42 percent). In the cefaclor group (67 patients) colonization occurred with potential pathogens in only 2 patients (3 percent). Superinfection occurred within six weeks of therapy in five patients in the amoxycillin group; no superinfections were observed in the cefaclor group (39). Therefore, use of antibiotics that impair CR may result in superinfections in general practice.

Classifying Micro-Organisms According To Intrinsic Pathogenicity

On the basis of the above, we may classify micro-organisms into three classes: pathogenic, potentially pathogenic, and low- or non-pathogenic.
Pathogenic micro-organisms overcome resistance to infection in healthy people and may cause epidemics among the normal population (diphtheria, pertussis, etc.) Prevention by means of vaccination may be indicated.
Potentially pathogenic micro-organisms cause infections only when infection resistance is impaired. The chance of infection is increased if organisms are present in high concentrations due to impairment of CR. Urinary tract infections are usually caused by *E. coli* and enterococci (primary endogenous flora of the intestine), respiratory infections by *S. pneumoniae, H. influenzae*, and *B. catarrhalis* (primary endogenous flora of the oropharynx), (40). When CR has been impaired, other potentially pathogenic micro-organisms, such as *Proteus, Klebsiella, Enterobacter*, and *Pseudomonas* spp. (secondary endogenous aerobic flora) may colonize the intestine and produce infection (secondary endogenous infection), (7,34).
Low- or non-pathogenic micro-organisms rarely cause infections, even if present in relatively large numbers and when resistance to infection is impaired. A typical example is the anaerobic flora that is autochthonous to the intestinal tract.

Selective Decolonization

Selective decolonization is the elimination of potentially pathogenic aerobic flora from the body without disturbing autochthonous anaerobic flora. Originally this was called "selective decontamination". However, because "decontamination" suggests the elimination of micro-organisms from inanimate objects, we now prefer "decolonization" as the term for elimination of micro-organisms from the body.

Patients with cytostatic-induced leukopenia have such a serious reduction of resistance to infection that they may develop infections even in the presence of normal concentrations of primary aerobic flora. Initial attempts to protect these patients by suppressing both anaerobes and aerobes have failed due to superinfections with micro-organisms resistant to the suppressive therapy. In view of observations that nearly all infections in such patients are caused by aerobic micro-organisms, and that CR is promoted by the presence of autochthonous anaerobic flora, it is logical to attempt selective decolonization with antibiotics that eliminate aerobic flora only (1).

A great number of studies have been published on the effect of selective decolonization of the lower digestive tract in leukopenic patients. These have been summarized elsewhere, and in general a two- to threefold reduction in risk of bacterial infection was found (1). Further reduction in risk of infection may be obtained by broadening the spectrum of the antibiotic combination used (41). Additional improvement may be possible *via* decolonization of the oral cavity, which can be achieved by using sticky oral paste or lozenges containing antimicrobial agents (1). The optimal regimen in leukopenic patients also requires decolonization of warm meals in a magnetron (microwave) oven (1), and exclusion of raw vegetables (12).

A novel application of selective decolonization is long-term prophylaxis of infection in patients on artificial ventilation. In these patients, the presence of a naso-tracheal canula reduces resistance to colonization of the upper respiratory tract as well as the resistance to contamination of the lower respiratory tract. Respiratory infections are common in these patients and mortality is high (2). At the hospital of the University of Groningen in the Netherlands, selective decolonization was employed in these patients, using non-

absorbable agents. The mouth was decolonized with an oral paste containing 2% concentrations of tobramycin, polymyxin B, and amphotericin B. Gastric and intestinal decolonization were achieved by administering the same nonabsorbable antibiotics through a stomach tube. In case of infection, only antibiotics that were presumed not to impair CR were used. This resulted in a marked decrease in infections due to Enterobacteriaceae. However, early respiratory infections (through day 3) by primary aerobic oropharyngeal flora (*S. pneumoniae* and *H. influenzae*) and nasopharyngeal flora (*S. aureus*) did not decrease (7). Therefore, in the succeeding patients cefotaxime 1 g iv qid was added to the regimen, starting from the time of intubation until potentially pathogenic bacteria had been eliminated from the oropharynx, usually within four days. The number of early infections was markedly reduced with this regimen. A decrease in incidence of infection from 81 to 16 percent among trauma patients requiring mechanical ventilation was observed in this study (42).

At the Canisius-Wilhelmina hospital, all patients on artificial ventilation are selectively decolonized with a less expensive regimen consisting of a mouth paste containing 2% norfloxacin, colistin and amphotericin B, and oral administration of norfloxacin 50 mg qid, amphotericin B 500 mg qid, and trimethoprim/sulfamethoxazole 125/125 mg qid. In an analysis of ongoing surveillance data, it was shown that the incidence of infection in patients on artificial ventilation was reduced from 75 to 30 percent. The number of Gram-negative infections fell six-fold and the incidence of other infections did not increase. At both centres septicemia is rare since the introduction of selective decolonization (42, 43).

We performed a prospective three-group randomized trial to assess the effect of selective decolonization and systemic antibiotic prophylaxis in patients on mechanical ventilation for at least five days. Preliminary results have been published. Patients in both control groups I (n=18) and II (n=21) did not receive antibiotic prophylaxis, but differed in the antibiotic policy in case of infection. Group I received antibiotic therapy known to disturb CR and group II was treated with antimicrobials presumed not to disturb CR. Patients in group III (n=17) received a prophylactic antimicrobial regimen designed to eliminate Gram-negative bacilli and yeasts from the digestive tract from the time of intubation until extubation. The

mouth was decolonized with an oral paste containing 2% tobramycin, colistin, and amphotericin B. One gram of this paste was applied to the oral mucosa four times daily. Gastric and intestinal decolonization were attempted by administration of colistin 200 mg, norfloxacin 50 mg, and amphotericin B 500 mg qid *via* the gastric tube (gastric suction was discontinued for one hour after administration). Systemic antimicrobial prophylaxis in group III patients consisted of cefotaxime 500 mg iv tid for five days.

Fourteen patients in group I (78 percent) and 13 patients in group II (62 percent) acquired at least one lower respiratory tract infection, with several acquiring more than one. A total of 25 infections were observed in the 14 patients in group I and 22 infections in the 13 patients in group II. Of these 47 infections , 43 (91 percent) were preceded by colonization of either the oropharynx, the stomach, or both with the causative micro-organisms. In group III, only one patient (6 percent) acquired a respiratory tract infection ($P=0.0001$). This infection (by *Serratia marcescens*) was not preceded by colonization of either the oropharynx or stomach. This study confirms the importance of gastrointestinal colonization in the etiology of respiratory infections, and the feasibility of preventing these infections by means of selective decolonization and short-term systemic prophylaxis.

Classifying Antimicrobial Agents According to Influence on Colonization Resistance.

Data citing the influence of antibiotics on CR are shown in the Table. Classification is based on data from healthy volunteers and from patients.

Antibiotics are classified as disturbing CR if studies in volunteers or patients show overgrowth of Gram-negative bacilli, enterococci, or yeasts, or if superinfections due to these organisms are observed. Antibiotics classified as not disturbing CR do not increase the concentration of Gram-negative bacilli, enterococci, or yeasts. Cefoperazone, for example, eliminates both aerobic Gram-negative rods and aerobic Gram-positive cocci from the bowel, but the concentration of yeasts is greatly increased (29). This shows that cefoperazone decreases CR. Quinolones, on the other hand,

Table. Classification of antimicrobial agents according to their influence on colonization resistance.

Antimicrobial agent (reference)	Disturbance of CR[a] : Yes	Unknown	No
Penicillins			
amoxycillin (9,39)	+		
ampicillin (35)	+		
benzylpenicillin			
($<3.10^6$ units/d, 44)			+
($>3.10^6$ units/d, 44)	+		
oxacillin (45)	+		
pheneticillin (46)	+		
piperacillin (29)	+		
Cephalosporins			
cefaclor (5, 46)			+
cefoperazone (29)	+		
cefotaxime		+	
cefoxitin (29)	+		
ceftazidime		+	
ceftriaxone (47)	+		
cefuroxime		+	
cephradine 1.5 g/d (1)			+
cephradine 6 g/d (1)	+		
Other agents			
chlortetracycline (1)	+		
clindamycin (31)	+		
colistin (49)			+
doxycycline (26, 46)			+
erythromycin (46)			+
quinolones (30)			+
tetracycline (48)	+		
co-trimoxazole (1, 46)			+

[a] colonization resistance

eliminate aerobic Gram-negative rods from the bowel and yield a temporary decrease in the concentration of enterococci.
The concentration of yeasts is unchanged or only marginally increased (30). Therefore, it is improbable that quinolones disturb anaerobic microbial CR.
The absence of activity against anaerobes might explain the lack of effect on CR noted with the sulphonamides, polymyxins, quinolones, and trimethoprim.
Similarly, the anti-anaerobic activity of beta-lactam antimicrobials might explain why penicillins and cephalosporins generally affect the CR, especially when given in high doses. Cephradine and cefaclor are exceptional, probably because they are well-absorbed and are eliminated or degraded so rapidly that they do not reach the CR-flora in the colon and the oropharynx in sufficient concentration. However, dosage may be a limiting factor, as it has been shown that cephradine 6 g/d disturbs CR in at least 50 percent of patients (1).
Tobramycin does not disturb CR when administered orally in doses of up 300 mg/d (50). Gastrointestinal excretion of tobramycin after parenteral administration is low and therefore tobramycin will probably not disturb CR when given in this way. Tetracyclines are apt to cause superinfections, possibly because of their activity on anaerobes together with incomplete absorption. However, a well-absorbed tetracycline like doxycycline does not disturb CR (26, 46).
Erythromycin has been used for selective decolonization of the intestinal tract and can be used safely for therapy, as can roxithromycin (9).
Tinidazole yields very low concentrations in faeces and does not seem to disturb CR when used in low doses, in contrast to clindamycin (32). Metronidazole may be comparable with tinidazole with respect to its influence on CR. Therefore, nitro-imidazoles are the preferred agents for therapy of anaerobic infections.

Implications for Antibiotic Policy

Most infections of the respiratory tract are caused by micro-organisms first colonizing the oropharynx (7, 14), whereas urinary tract infections are caused by micro-organisms first colonizing the intestinal tract (51) or vagina (13). The risk of infection may

increase with the concentration of colonizing micro-organisms. Therefore, administration of antibiotics that impair CR should be avoided because this may increase the number of superinfections.
Effective treatment together with elimination of flora at the site of colonization will reduce the risk of re-infection (51). Even in the presence of normal concentrations of potentially pathogenic micro-organisms, endogenous infections may occur when infection resistance is reduced by disease (e.g., leukopenia) or medical interventions (e.g., endotracheal intubation or transurethral catheterization). In these clinical settings, development of endogenous infections may be prevented by selective decolonization (7, 17, 52). For suppression of micro-organisms in the throat, an oral paste or lozenge should be used; suppression of gastrointestinal flora may be achieved by administering antibiotics by mouth or *via* gastric tube.
In summary, we recommend the following policy :
(1) If the effectiveness, toxicity, and price of antibiotics are equivalent, an agent that does not impair CR is preferred.
(2) When possible, use an antibiotic that will both eradicate the infection and provide selective decolonization.
(3) Selective decolonization of the oropharynx and gastrointestinal tract is indicated in all patients with markedly reduced resistance to infection.

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CHAPTER III

INFLUENCE OF AMOXYCILLIN, ERYTHROMYCIN AND ROXITHROMYCIN ON COLONIZATION RESISTANCE AND ON APPEARANCE OF SECONDARY COLONIZATION IN HEALTHY VOLUNTEERS

E.J. Vollaard[a], H.A.L. Clasener[b], A.J.A. van Griethuysen[b], A.J.H.M. Janssen[b] and A.H.J. Sanders-Reijmers[b]

Departments of [a]Pharmacy and [b]Microbiology, Canisius-Wilhelmina Hospital, St. Annastraat 289, 6525 GT Nijmegen, The Netherlands

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Abstract

We investigated the influence of oral administration of amoxycillin, erythromycin and roxithromycin on colonization resistance (CR) in healthy volunteers. Antibiotics were administered in a randomized cross-over design.
No effect on the CR of the oropharynx could be demonstrated.
Amoxycillin decreased the CR of the bowel against Gram-negative bacilli and yeasts, whose median concentration in faeces increased 100-fold and 30-fold respectively. Roxithromycin and erythromycin decreased the concentration of Gram-negative bacilli in faeces.
Secondary colonization with Gram-negative bacilli was detected as often following roxithromycin as following amoxycillin, but the level of colonization with these bacteria was much higher following amoxycillin.
Following roxithromycin and erythromycin the level of secondary colonization did not exceed the original concentration of Gram-negative bacilli, showing that these antibiotics did not decrease the CR against Gram-negative bacilli.
The appearance of secondary colonization in faeces at levels equal to or lower than the concentration of Gram-negative bacilli before administration of antibiotics, should not be regarded as proof of disturbance of CR.

Introduction

As early as 1947, it was suggested that superinfections following the administration of streptomycin and penicillin are caused by their influence on the microbial flora of the digestive tract (1). It was later noted that some antibiotics caused tremendous overgrowth of minor microbial groups of the intestinal flora, increasing the total aerobic flora by 100-fold to 1000-fold (2). In mice, oral streptomycin greatly increases susceptibility to intestinal colonization and subsequent infection by salmonellae (3, 4).
From studies of the effect of oral penicillin, tetracycline and chloramphenicol on the composition of the faecal flora of mice, and from morphological studies of the gut of germ-free mice, it was concluded that the normal intestinal flora is made up of two completely

different classes of micro-organisms : (1) autochthonous symbiotic flora consisting of anaerobic bacteria in high numbers, and (2) potentially pathogenic flora consisting of aerobic bacteria in much lower, variable numbers. The autochthonous flora is essential for normal development of the gut, and has a protective function by limiting the number of potentially pathogenic micro-organisms (5, 6).
In 1971, the limiting action of autochthonous flora on the potentially pathogenic flora was rediscovered and named CR (7). Antimicrobial agents that decrease CR promote superinfections in two ways : (1) by lowering the dose of potentially pathogenic micro-organisms that is required for colonization of the intestinal tract, and (2) by allowing potentially pathogenic micro-organisms to attain high concentrations in the digestive tract.
Antimicrobial agents that do not decrease CR have been used for a method of long-term prophylaxis of endogenous infections in leukopenia and in mechanical ventilation, called 'selective decontamination' (8). This method consists of elimination of the digestive tract as a source of potentially pathogenic micro-organisms, while sparing the autochthonous flora.
Although new antimicrobial agents appear abundantly, there is still a shortage of those that do not affect CR, not only for selective decontamination, but also for antimicrobial therapy that does not provoke superinfections.
Therefore we took the opportunity to test the effect of roxithromycin on CR. Roxithromycin resembles erythromycin which has been claimed to be useful in selective decontamination (9). Apart from use in selective decontamination, roxithromycin might be useful for therapy of respiratory infections. In mice roxithromycin and erythromycin do not affect CR (10).
In our study roxithromycin is compared with erythromycin and with amoxycillin, which may decrease CR (11, 12).
Influence of antimicrobial agents on CR can be studied best by measurement of their effects on the concentration of potentially pathogenic micro-organisms, because the anaerobic flora responsible for CR is still unknown.
In this study, increase in the faecal concentration of Gram-negative bacilli will be considered as the most important indication of decrease of CR. Other parameters thought possibly useful are the appearance of Gram-negative bacilli in the oropharynx and the

appearance of new species of Gram-negative bacilli in faeces, referred to as secondary colonization.
Studies in healthy volunteers are to be preferred, because CR may also be decreased by illness, (13, 14). A cross-over design was chosen to compensate for interindividual differences in CR.

Methods

Study design
Healthy volunteers received one of the three study drugs, in a randomized cross-over study design, for seven days. This was followed by a 21-day wash-out period, after which the next treatment with another of the study drugs started.
Oropharyngeal gargling samples (20 ml physiological saline) and faecal samples were taken on the morning of day 1 (before the first antibiotic dose), 4, 8, 15, 22 and 29. In the week before the start of the study two extra samples were taken, at days -7 and -4.
Permission for this study was obtained from the ethics committee of the Canisius-Wilhelmina Hospital.

Volunteers
Twelve healthy normal volunteers (four female, eight male), aged 23 to 49 (median 36) participated in the study. Written informed consent was obtained. The volunteers had not used antibiotics for at least one month before the start of the study.

Drugs
Amoxycillin (Clamoxyl, Beecham) 500 mg capsules were administered three times daily. Erythromycin stearate (Erythrocine, Abbott) 500 mg tablets were administered twice daily. Roxithromycin (Roussel) 150 mg tablets were administered twice daily. All drugs were given at the end of a meal.

Bacteriology
Serial 1/10 dilutions of faeces were made in Thioglycollate medium (BBL). One-microlitre volumes of each dilution were inoculated on to solid media, selective for, respectively, Gram-negative bacilli (Eosin-methylene-blue lactose sucrose agar, Merck) ; for enterococci

(5% sheep blood in blood agar base (Oxoid) with nalidixic acid 50 mg/l) ; for *Staphylococcus aureus* (Mannitol salt agar (bio-Merieux); and for yeasts (Sabouraud dextrose agar (Gibco) with chloramphenicol 125 mg/l. The solid selective media were also inoculated with 100-microlitre volumes of the first 1/10 dilution of faeces. Concentrations were expressed as the logarithms to the base of 10 of the counts per gram of faeces, rounded up or down to whole numbers.
Gargling fluids were inoculated in volumes of 100 microliter on the selective media.
Micro-organisms isolated were identified and antibiotic sensitivity profiles were determined by standard laboratory methods.

Statistics
Friedman's test was employed to evaluate the differences in concentration of micro-organisms in faeces following administration of antibiotics

Results

Two volunteers missed one study period because of intercurrent illness. They were excluded from evaluation.

Gargling fluid

Yeasts were never detected in gargling fluid samples of four volunteers, and in 2, 2, 3, 5, 9, and 15 of 18 samples in the other volunteers. No relation with administration of antibiotics was detected, except in one volunteer, who had four positive cultures following amoxycillin and roxithromycin each, out of 9/18 positive cultures.
S. aureus was never detected in gargling fluid samples of four volunteers and in 1, 4, 4, 7, 8 and 13 of 18 samples in the other volunteers. No relation with antibiotic administration was observed.
Gram-negative bacilli were detected in gargling fluid samples of only two volunteers in 5 and 8/18 samples respectively. No relation with antibiotic administration was observed.

Yeasts

The median count of yeasts was 10^2 cfu/g (range $< 10^2$ cfu/g - 10^5 cfu/g). No differences were seen following adminstration of erythromycin ($P = 0.57$) and roxithromycin ($P = 0.73$), but following amoxycillin an increase to $10^{3.5}$ at day 8 ($P < 0.001$) was observed.

S. aureus

S. aureus was detected in 15/180 faecal samples. No relation with antibiotics could be found.

Enterococci

Enterococci were present in a median count of 10^5 cfu/g (range 10^2 cfu/g - 10^8 cfu/g). They were suppressed to a level of $10^{3.5}$ cfu/g on day 8 by erythromycin ($P = 0.02$) and amoxycillin ($P = 0.09$), but not by roxithromycin ($P = 0.98$).

Gram-negative bacilli

1. Total level of colonization in faeces.

During administration of amoxycillin the median concentration of Gram-negative bacilli increased from 10^6 cfu/g before administration to 10^8 cfu/g following one week of amoxycillin ($P < 0.01$, Figure 1). The concentration of Gram-negative bacilli in faeces increased in eight volunteers and remained unchanged in two volunteers.

Erythromycin and roxithromycin decreased the median concentration of Gram-negative bacilli from 10^7 cfu/g to 10^2 cfu/g and from 10^6 cfu/g to 10^3 cfu/g respectively ($P < 0.001$ in both cases), Figures 2 and 3). Following both erythromycin and roxithromycin the concentration of Gram-negative bacilli decreased in nine volunteers and increased 1 $\log_{10}$ in one volunteer.

One week after each drug normal concentrations of Gram-negative bacilli were found again.

2. Secondary colonization with Gram-negative bacilli.

Gram-negative bacilli other than *Escherichia coli* (secondary Gram-negative bacilli) were not detected in any of the 20 pre-study samples and in only one of the 30 faecal samples taken at day 29 of the antibiotic periods.

Antibiotic administration increased detection of secondary colonization (Figure 4), which was caused most often by *Klebsiella* species (Table I). On day 8, secondary colonization was observed in 5/10

volunteers following amoxycillin and roxithromycin, and in 1/10 volunteers following erythromycin. Following amoxycillin the counts were much higher (10^6 cfu/g - 10^{10} cfu/g) than following roxithromycin (10^3 cfu/g - 10^7 cfu/g, Figure 4).
Following amoxycillin high counts of secondary Gram-negative bacilli were observed in the presence of high counts of *E. coli*. During administration of roxithromycin, secondary colonization occurred in 3/5 volunteers after disappearance of *E. coli* (day 4 or 8), and ended when or shortly after *E. coli* had reached its original concentration (day 15 - 22, Table II). Following erythromycin few cases of secondary colonization were detected. All secondary colonizing Gram-negative bacilli were resistant to the antibiotic administered at the time of isolation.
Disappearance of secondary colonization was much slower than normalization of the total level of colonization with Gram-negative bacilli (Figures 1 to 4).

Discussion

This study confirms the negative influence of amoxycilin on colonization resistance of the bowel (11, 12). In contrast to van Saene *et al.*, (15), we found no disturbance of CR of the oropharynx by amoxycillin. Their study, however, was not done in volunteers but in patients.
Erythromycin and roxithromycin decreased the level of Gram-negative bacilli in faeces, but did not eliminate them. Thus in the prophylaxis of patients with very low infection resistance, such as those with leukopenia, they should be combined with a second decontaminating agent.
Our results with erythromycin are in agreement with those of Andremont (9, 16) and Hartley (17). However, Heimdahl and Nord (18) claim major disturbance of the aerobic faecal flora in volunteers following erythromycin stearate 500 mg twice daily for seven days. They found secondary colonization of the bowel with Gram-negative bacilli in 6/10 volunteers, but not exceeding 10^5 cfu/g of faeces. So in our view CR of the bowel was not disturbed. In contrast to our findings, they also found colonization of the oropharynx with Gram-negative bacilli in 7/10 volunteers, in a concentration of 10^3 to 10^8

cfu/ml, showing disturbance of the CR of the oropharynx. This difference with our results is hard to explain. It should be noted, however, that our study followed a cross-over design.
Secondary colonization with Gram-negative bacilli was observed as often following amoxycillin as following roxithromycin. In contrast to amoxycillin, roxithromycin did not cause secondary colonization in concentrations exceeding the normal concentration of Gram-negative bacilli in the volunteers concerned. We therefore conclude that roxithromycin did not disturb the CR against Gram-negative bacilli.

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Mrs A. Remie-Wijlhuizen, Mrs M.P.W.P. van Gemert-Toonen and Mr C.A. van Veen provided technical assistance, Mrs L. Janssen-Lamers typed the manuscript and Roussel Laboratoria BV, the Netherlands, provided financial support.

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Table I. Secondary colonization of faeces with Gram-negative bacilli following antibiotics

Micro-organism	Number of samples positive[a] during : amoxycillin period(60)[b]	erythromycin period(60)[b]	roxithromycin period(60)[b]	total
K. pneumoniae	10	2	5	17 (42.5%)
K. oxytoca	2	1	8	11 (27.5%)
K. group 47	1	-	-	1 (2.5%)
E. cloacae	1	1	1	3 (7.5%)
E. agglomerans	1	-	1	2 (5.0%)
H. alvei	1	1	1	3 (7.5%)
C. freundii	-	-	1	1 (2.5%)
S. liquefaciens	-	-	1	1 (2.5%)
UGNB[c]	1	-	-	1 (2.5%)

[a] : Some samples contained more than one species.
[b] : Number of samples examined.
[c] : Unidentified Gram-negative bacilli.

Table II. Concentration of E. coli and other Gram-negative bacilli in five volunteers following administration of roxithromycin

Volunteer	Micro-organism	Log_{10} concentration on day : 1	4	8	15	22	29
a	E. coli	6	4	4	7	7	6
	K. pneumoniae	-	-	4	3	-	-
b	E. coli	7	3	-	5	6	7
	E. agglomerans	-	-	3	-	-	-
c	E. coli	8	-	-	6	6	6
	K. oxytoca	-	6	5	6	5	-
d	E. coli	7	3	-	7	7	8
	3 species	-	-	3	7	-	-
e	E. coli	6	7	7	8	7	7
	2 species	-	7	7	7	6	-

- : Below detection limit (10^2 cfu/g).

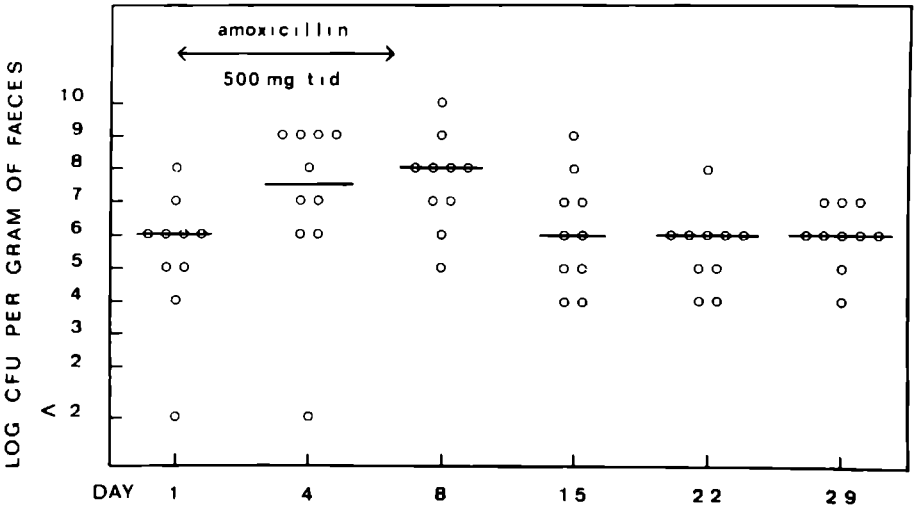

Figure 1. Concentration of Gram-negative bacilli in faeces of ten volunteers following oral amoxicillin 500 mg tid.
—— : Median concentration

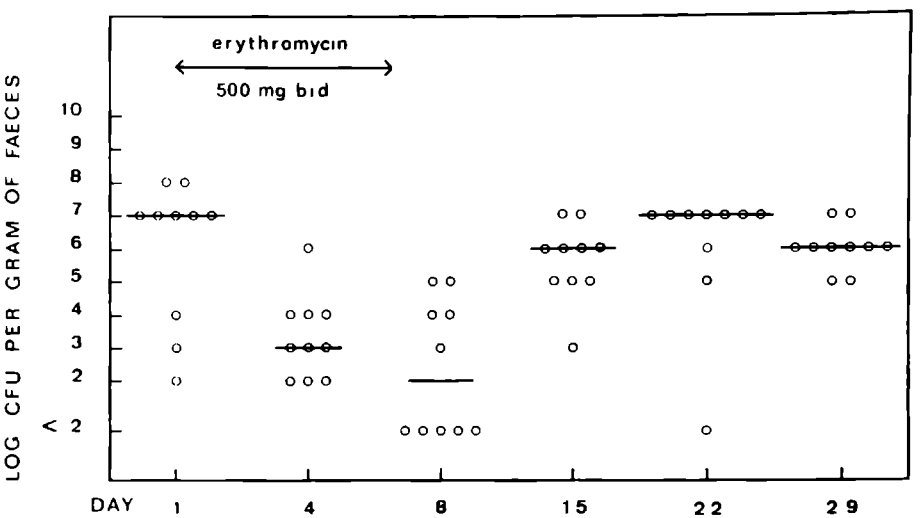

Figure 2. Concentration of Gram-negative bacilli in faeces of ten volunteers following oral erythromycin 500 mg bid.
—— : Median concentration.

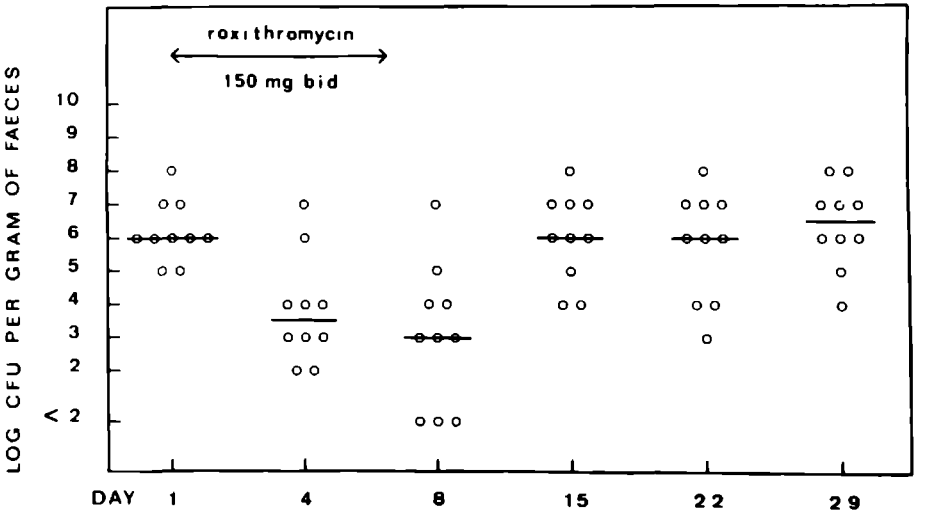

Figure 3. Concentration of Gram-negative bacilli in faeces of ten volunteers following oral roxithromycin 150 mg bid.
—— : Median concentration.

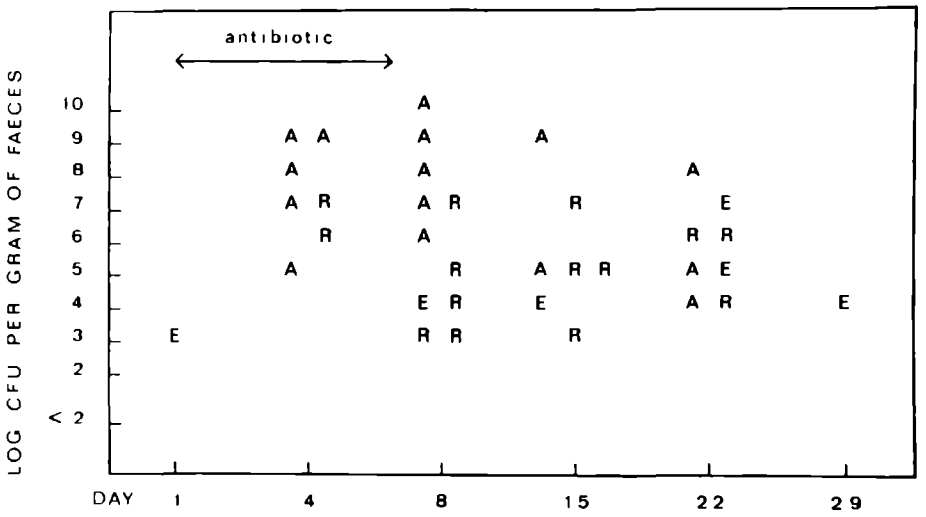

Figure 4. Concentration of secondary Gram-negative bacilli in faeces of ten volunteers following oral amoxycillin (A), erythromycin (E), or roxithromycin (R).

CHAPTER IV

INFLUENCE OF CEFACLOR, PHENETHICILLIN, CO-TRIMOXAZOLE AND DOXYCYCLINE ON COLONIZATION RESISTANCE IN HEALTHY VOLUNTEERS

E.J. Vollaard[a], H.A.L. Clasener[b], A.J.A. van Griethuysen[b], A.J.H.M. Janssen[b], A.H.J. Sanders-Reijmers[b], N.F. Muller[a] and P.J.C. Huige[c]

Departments of [a]Pharmacy and [b]Microbiology, Canisius-Wilhelmina Hospital, St. Annastraat 289, 6525 GT Nijmegen, The Netherlands
[c]Eli Lilly, The Netherlands

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Abstract

The influence of oral administration of cefaclor, phenethicillin, co-trimoxazole and doxycycline on colonization resistance (CR) of the oropharynx and of the colon was studied in healthy volunteers. Antimicrobial agents were administered in a randomized cross-over design. No effect on CR of the oropharynx could be demonstrated. Phenethicillin decreased CR of the colon against Gram-negative bacilli ($P = 0.001$). Co-trimoxazole significantly decreased the concentration of Gram-negative bacilli in faeces ($P = 0.03$), but the decrease caused by cefaclor and doxycycline did not reach statistical significance. Administration of antimicrobial agents increased the appearance of secondary colonization by Gram-negative bacilli in faeces, especially when *Escherichia coli* was eliminated. During administration of phenethicillin, secondary colonization occurred at a concentration exceeding 10^7/g in some volunteers. Following administration of cefaclor, co-trimoxazole and doxycycline, elimination of *E. coli* may result in the substitution by resistant Gram-negative bacilli in low concentration.

Introduction

An important problem following the use of antimicrobial agents is the appearance of resistant species and the occurrence of super-infections. One solution to this problem is by advocating the restriction of antibiotic usage, and stressing the importance of hygienic measures. The prevention of resistant micro-organisms appearing at all would seem to be a more promising approach. According to the theory of colonization resistance (CR), suppression of a certain (anaerobic) part of the indigenous flora will promote colonization of the digestive tract by resistant micro-organisms at high concentrations, thereby increasing the source and thus the risk of cross-contamination and of superinfections. On the other hand, antimicrobial agents that do not disturb anaerobic indigenous flora and reach sufficiently high concentrations in the digestive tract, will achieve 'selective decontamination' of potentially pathogenic aerobic flora. Such agents have been used successfully for the prevention of infection in neutropenic patients and in those on mechanical

ventilation (1). Therefore, antimicrobial agents to be used for therapy or prophylaxis need to be investigated for their effects on the indigenous flora.

Antimicrobial agents may influence the microbial flora of the digestive tract when incompletely absorbed following oral administration, or when excreted in saliva, bile or mucus (2).

In the intestine antibiotics are inactivated to a variable extent by decomposition (3, 4), or by binding (5, 6, 7). The remaining active antimicrobial agent may eliminate the susceptible part of the indigenous flora. In mice which have been pre-treated with streptomycin (8, 9) or streptomycin and neomycin (10) the infective dose of exogenous micro-organisms is between 1000-fold and 100,000-fold lower than in untreated controls. In mice which have been treated with streptomycin (9), penicillin (11) or ampicillin (12), or streptomycin and neomycin (13), and in germfree mice (14, 15), the concentration of aerobic Gram-negative challenge strains in faeces becomes between 1000-fold and 100,000-fold higher than the normal level of *E. coli* (c.10^5/g) found in faeces.

In man also, the administration of antimicrobial agents may result in overgrowth by resistant, potentially pathogenic aerobic flora and in decrease of the infective dose of enteric pathogens (16, 17, 18, 19). Conversely, in germfree mice (14) and in antibiotically decontaminated mice (13), the abnormally high susceptibility to colonization by aerobic micro-organisms may be strongly reduced by administration of murine or even human anaerobic flora (15). In man, a dramatic cure was reported in patients suffering from pseudomembranous colitis when fresh faecal suspensions were administered by an intrajejunal tube or by enemas (20, 21).

The protective effect of indigenous flora against new colonization by exogenous micro-organisms has originally been called colonization resistance (CR) (10). The term CR has also been used to indicate the limiting action of indigenous flora on the concentration of potentially pathogenic aerobic flora in the intestine (22). The CR flora is probably mainly anaerobic, but the exact composition remains unknown (1). Consequently it is impossible to predict the influence of antimicrobial agents on CR by *in-vitro* determinations of the susceptibility of known anaerobic species. Therefore, the influence of antimicrobial agents on CR should be investigated by their administration to animals or humans, and measuring their influence

on the concentration of aerobic micro-organisms in faeces and on the appearance of Gram-negative bacilli and yeasts in the oropharynx. Preferably, these investigations should be performed in humans, because dose-response effects of antimicrobial agents on CR in animals are difficult to extrapolate to man. In patients, factors other than the microbial CR, such as salivation, swallowing, and peristalsis, may be disturbed. Consequently healthy volunteers are more suitable.

In comparing the influence of different antibiotics on CR, a cross-over study design is preferable, to compensate for inter-individual differences in normal CR. In this study, we investigated the influence of cefaclor, phenethicillin, co-trimoxazole and doxycycline in human volunteers. Cefaclor, 250 mg three times daily, does not disturb CR in human volunteers (23). In patients with upper airway infections, cefaclor 250 mg three times daily did not provoke superinfections, in contrast to amoxycillin 375 mg three times daily (24). In our study a higher dosage of cefaclor was investigated. Phenethicillin is suspected of disturbing CR, because penicillin at high dosage is known to cause superinfections (25). Doxycycline is supposed to have little influence on CR (26), whilst co-trimoxazole is used in the selective decontamination of neutropenic patients (1).

Increase in faecal aerobic flora was considered as the most important indication of a decrease in CR of the bowel. Detection of Gram-negative bacilli in gargling fluid was considered as an indication of decrease in CR of the oropharynx. Another parameter thought possibly useful was the appearance of new species of Gram-negative bacilli in faeces, referred to as secondary colonization.

Methods

Study design

Healthy volunteers received one of the four drugs in a randomized cross-over study for seven days, followed by a 14-day wash-out period, after which the next treatment with another of the study drugs started. Oropharyngeal gargling samples (20 ml of physiological saline) and faecal samples were taken on the morning of days 1 (before the first antibiotic dose), 4, 8, 11, 15 and 22. Permission for the study was obtained from the ethics committee of

the Canisius-Wilhelmina Hospital.

Volunteers
Twelve healthy normal volunteers (4 female, 8 male), age 22 to 52 (median age 31), participated in the study. Written informed consent was obtained. The volunteers had not used antibiotics for at least one month prior to the start of the study.

Drugs
Cefaclor 500 mg capsules and phenethicillin 500 mg capsules were used three times daily. Co-trimoxazole 960 mg capsules were administered two times daily and doxycycline 100 mg capsules were administered once daily.
The study was conducted in a double blind design, with identically looking capsules containing study drugs and placebos. The volunteers received separate bottles for the morning, noon and afternoon doses. Placebos were used for the noon dose of co-trimoxazole and for the noon and afternoon dose of doxycycline. All drugs were taken at the end of a meal.

Bacteriology
Serial 1/10 dilutions of faeces were made in Thiogycollate medium (BBL). One-microlitre volumes of each dilution were inoculated on to solid media to isolate Gram-negative bacilli (Eosin methylene-blue lactose sucrose agar, Merck), enterococci and viridans streptococci (5% sheep blood in agar base, Oxoid, with nalidixic acid 50 mg/l), *Staphylococcus aureus* (Mannitol salt agar, bioMérieux), and yeasts (Sabouraud dextrose agar, Gibco, with chloramphenicol 125 mg/l). The solid media were also inoculated with 100-microlitre volumes of the first 1/10 dilution of faeces, lowering the limit of detection to 100 micro-organisms per gram of faeces. Concentrations were expressed as the logarithms to the base 10 of the counts per g of faeces, rounded up or down to whole numbers. Gargling fluids were inoculated in volumes of 100-microlitre on to the solid media. Micro-organisms isolated were identified by standard laboratory methods.

Statistics
Friedmans's test was employed to evaluate the differences in counts

of micro-organisms in faeces following administration of antibiotics. The counts in samples on the first day of the study were used as a baseline.

Results

Two male volunteers missed one study period because of intercurrent illness. They were excluded from analysis. Results in the remaining ten volunteers are presented.

Gargling fluid

Yeasts were not detected in any sample from five volunteers and in 2, 2, 2, 11, and 12 samples out of 21 samples in the others. Counts did not exceed 10^3/ml of gargling fluid. No obvious relation with administration of antibiotic was seen. *S. aureus* was not detected in nine volunteers. In the tenth, one sample was positive (10^2/ml). Strains of Gram-negative bacilli were detected in 1, 1, and 3 samples from three volunteers respectively. The counts were 10^2/ml in all cases. The three positive samples from one volunteer occurred on days 11, 15 and 22 of the doxycycline period.
Counts of viridans streptococci were 10^5 - 10^7/ml in all 21 samples from four of the volunteers. In the others few samples showed counts outside this range.

Faeces

Yeasts
At the start of the study, culture for yeasts was negative in 6/10 volunteers. Two volunteers carried 10^2 yeasts per g faeces, one 10^3, and one 10^5. No significant differences were observed at the end of each study period.
S. aureus
S. aureus was not detected in nine volunteers. The tenth showed two positive samples (both 10^3/g) on day 11 when receiving cefaclor and phenethicillin.

Enterococci

Before the start of antibiotics the median counts of enterococci were 10^4/g (range 10^4/g - 10^6/g). No significant differences were seen at the end of each study period.

Administration of cefaclor and doxycyclin had no significant effect, but co-trimoxazole and phenethicillin decreased counts to a median of 10^3/g (range < 10^2/g - 10^6/g, $P = 0.05$) and 10^4/g (range < 10^2/g - 10^7/g, $P = 0.03$) respectively.

Gram-negative bacilli

1. Total level of colonization in faeces.

The median level at day 1 of the study was 10^7/g (range 10^5/g - 10^8/g). At the end of the study periods (day 22) the count was slightly lower, especially after co-trimoxazole (10^5/g, $P > 0.05$, Figure 3). During administration of phenethicillin an increase to a median count of 10^8/g ($P = 0.001$) was observed. Administration of cefaclor and doxycycline led to a slight decrease to 10^5/g and 10^4/g respectively, but only with co-trimoxazole was the decrease (to 10^2/g) significant ($P = 0.03$) (Figures 1-4).

2. Secondary colonization with Gram-negative bacilli.

Gram-negative bacilli other than *E. coli* (secondary Gram-negative bacilli) were detected in two of 40 faecal samples taken on day 1 of the study periods. Antibiotic administration increased the detection of secondary colonization, which was most often caused by *Klebsiella* species (Table I). Following phenethicillin, secondary colonization appeared sooner after the start end disappeared sooner after the end of administration than was the case following doxycycline or co-trimoxazole. Following cefaclor, few cases of secondary colonization were noted. Following phenethicillin, the counts of secondary colonizing Gram-negative bacilli were much higher (10^5/g - 10^{10}/g) than those following the other antibiotics (10^2/g - 10^7/g) (Figure 5 and Table II).

Discussion

The principal indication of disturbance of CR is an increase in the concentration of aerobic flora in the digestive tract. According to this criterion, our study shows disturbance of CR of the lower parts of the intestinal tract by phenethicillin, but not by cefaclor, co-

trimoxazole or doxycycline.
This is in accordance with earlier investigations in both patients and human volunteers with cefaclor (23, 24), co-trimoxazole (27, 28) and doxycycline (26, 29). The influence of phenethicillin on CR in the human bowel confirms the findings in mice (30). Another indication of impairment of CR is that colonization of the digestive tract will take place following challenge with lower numbers of Gram-negative bacilli than are needed in the presence of normal CR. Therefore the occurrence of secondary colonization with Gram-negative bacilli has been considered an indication of a decrease in CR. However, increase in detection of secondary colonization using standard laboratory methods is not necessarily the same as an actual increase in the occurrence of secondary colonization. In this study 16/26 cases of secondary colonization were found in samples in which *E. coli* was undetectable (Table II). In the other positive samples the concentration of *E. coli* was equal to or only slightly different from the concentration of the secondary Gram-negative bacilli. Appearance of secondary colonization when *E. coli* concentration falls, can be explained by the fact that strains present in a concentration of more than 100 times less than that of the dominant *E. coli* strain, will seldom be detected unless selective media are used. Faecal colonization by *K. pneumoniae* at a level of 10^4 cfu/g will seldom be noticed in the presence of 10^7 cfu of *E. coli* per g in faeces. However, the presence of *K. pneumoniae* at this level will be detected following elimination of *E. coli* by a suitable antimicrobial agent. Thus, increased detection of secondary colonization following elimination of *E. coli* does not prove increased occurrence. The data in Table II suggest however that the decrease or elimination of *E. coli* might improve the detection of secondary colonization, as well as increase the occurrence of secondary colonization following administration of cefaclor, co-trimoxazole or doxycycline.
In animal experiments it has been shown that *E. coli* may play a role in the prevention or suppression of secondary colonization (31, 32). As long as the anaerobic flora is not impaired however, absence of *E. coli* did not result in secondary colonization in a concentration exceeding the normal level of *E. coli* in faeces (14). The same kind of bacterial interference probably exists in the human intestine, because in this and in a previous study (33) secondary colonization

at a level of 10^8 cfu/g or higher only occurred following amoxycillin and phenethicillin, suggesting disturbance of anaerobic flora. Following roxithromycin, erythromycin, cefaclor, co-trimoxazole and doxycycline however, secondary colonization did appear, but always at a level equal to or lower than the normal concentration of *E. coli* in the volunteers concerned, suggesting no disturbance of anaerobic flora.

Incomplete suppression of *E. coli* or increased occurrence of low concentrations of secondary Gram-negative bacilli, following elimination of *E. coli*, is probably not a dangerous consequence of an antibiotic in patients with normal infection resistance. In patients with very low infection resistance however, such as neutropenic patients, even the presence of low concentrations of potentially pathogenic micro-organisms in the digestive tract may result in infection. Therefore, in these patients the spectrum of the regimen should also cover secondary colonization. This may explain why the addition of colistin improves the results obtained by selective decontamination with co-trimoxazole alone (34), and why ciprofloxacin is superior to the combination of co-trimoxazole and colistin (35).

The four antibiotics tested had no effect on oropharyngeal flora as detected in gargling fluid. Concentrations of viridans streptococci did not change, and no increase in secondary colonization occurred. This study did not demonstrate that any of these antibiotics predispose to superinfections of the upper airways. It is possible though that volunteers are less susceptible than patients to the facilitation of secondary colonization of the oropharynx by antibiotics. In another study (33) we did not observe an increase in secondary colonization of the oropharynx following amoxycillin in volunteers. Both Lacey (36) and van Saene (24) however, showed an increase in secondary colonization of the oropharynx following amoxycillin in patients. These authors did not use gargling samples, as we did, but pharyngeal swabs. As it has been shown that the gargling method is more sensitive (37), this fact alone cannot explain the discrepancy between the results.

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Table I. Secondary colonization of faeces with Gram-negative bacilli day 4-22

Micro-organism	Number of samples positive[a] following: CFC (50)[b]	PTC (50)[b]	CTX (50)[b]	DXC (50)[b]	Total
K. pneumoniae	1	6		1	8
K. oxytoca	1	3	1		5
P. aeruginosa	1		3		4
P. putida			1		1
C. freundii				3	3
E. agglomerans			2		2
A. lwoffii			1		1
H. alvei			1		1
P. mirabilis				1	1
S. liquefaciens		1			1
S. marcescens		1			1
	--	--	--	--	--
Total	3	11	9	5	28

[a] : Some samples contained more than one species.
CFC : cefotaxime ; PTC : phenethicillin ; CTX : co-trimoxazole, DXC : doxycycline.
[b] : Number of samples examined.

Table II. Concentrations of E. coli and other Gram-negative bacilli in faeces in which secondary colonization appeared

Period and volunteer	Micro-organism	Log_{10} concentration on day : 1	4	8	11	15	22
Cefaclor							
B	E. coli	5	5	5	-	2	7
	K. oxytoca	-	-	-	2	-	-
H	E. coli	5	5	5	4	4	5
	P. aeruginosa	-	5	-	-	-	-
	K. pneumoniae	-	2	-	-	-	-
Co-trimoxazole							
C	E. coli	-	-	-	-	-	-
	K. oxytoca	-	-	-	-	-	4
H	E. coli	7	2	-	-	6	5
	P. aeruginosa	-	-	4	3	-	4
J	E. coli	5	5	-	-	-	7
	A. lwoffii	-	-	3	-	-	-
	H. alvei	-	-	-	-	2	-
K	E. coli	4	3	6	-	-	-
	P. putida	-	3	-	-	-	-
	E. agglomerans	-	-	-	6	4	-

- : Below detection limit (10^2 cfu/g).

(continued on next page)

Table II. Concentration of E. coli and other Gram-negative bacilli in faeces in which secondary colonization appeared (continued from preceding page).

Period and Volunteer	Micro-organism	Log_{10} concentration on day : 1	4	8	11	15	22
Doxycycline							
B	E. coli	7	3	7	-	6	7
	C. freundii	-	-	-	4	-	-
F	E. coli	6	6	4	7	8	7
	K. pneumoniae	6	-	-	-	-	-
	C. freundii	-	-	4	7	-	-
J	E. coli	7	3	-	-	-	7
	K. pneumoniae	-	-	-	3	-	-
K	E. coli	-	-	3	4	4	9
	P. mirabilis	-	-	-	-	4	-
Phenethicillin							
C	E. coli	5	-	-	-	-	-
	K. pneumoniae	-	5	5	5	-	-
	S. liquefaciens	-	-	5	-	-	-
	K. oxytoca	-	-	-	5	-	-
I	E. coli	6	8	7	7	5	4
	K. oxytoca	-	8	-	-	-	-
J	E. coli	7	-	7	6	6	9
	K. oxytoca	-	8	-	-	-	-
	K. pneumoniae	-	-	7	-	-	-
K	E. coli	8	7	-	7	4	6
	S. marcescens	8	-	10	-	-	-
	K. pneumoniae	-	-	10	-	-	-
L	E. coli	7	-	7	6	7	7
	K. pneumoniae	-	8	-	-	-	-

- : Below detection limit (10^2 cfu/g).

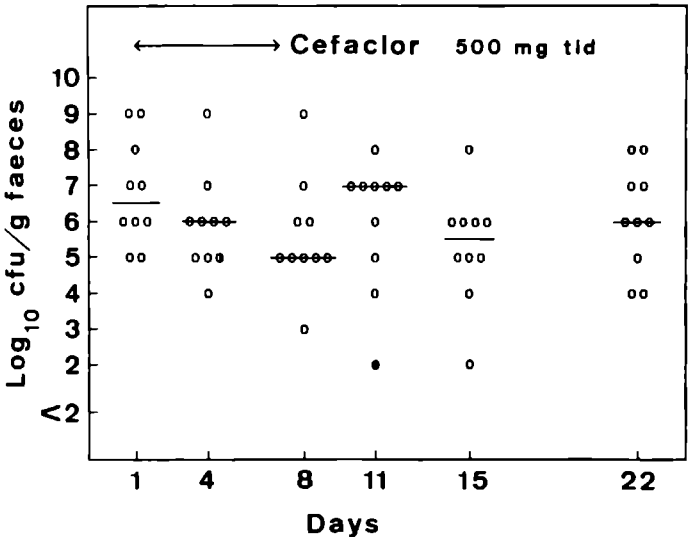

Figure 1. Faecal concentration of Gram-negative bacilli following cefaclor.
o : E. coli dominant.
● : Other Gram-negative bacilli dominant.
⊙ : E coli and other Gram-negative bacilli present in the same concentration.
—— : median concentration of Gram-negative bacilli.

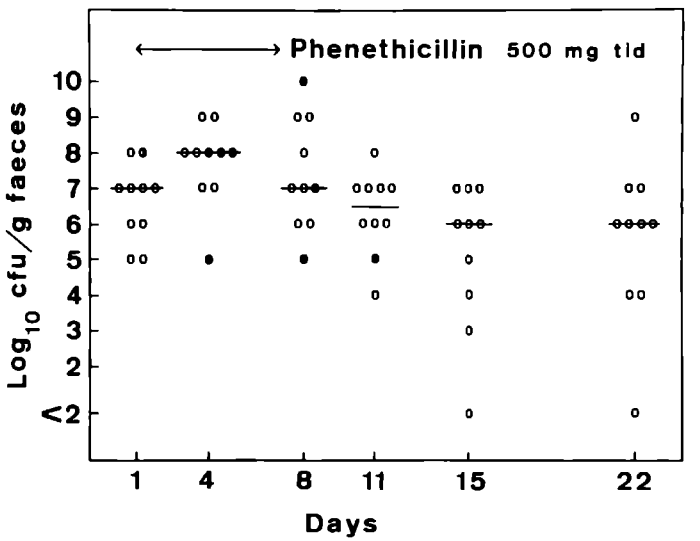

Figure 2. Faecal concentration of Gram-negative bacilli following phenethicillin.
Legends as in Figure 1.

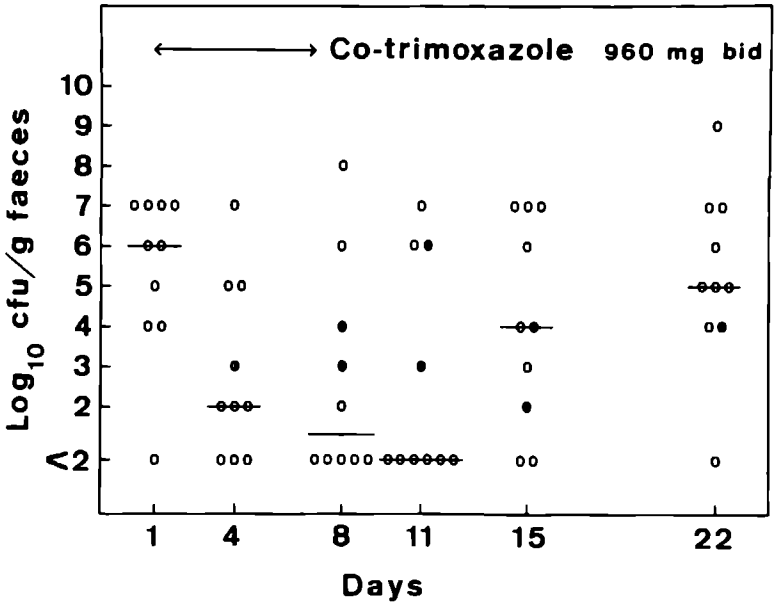

Figure 3. Faecal concentration of Gram-negative bacilli following co-trimoxazole.
Legends as in Figure 1.

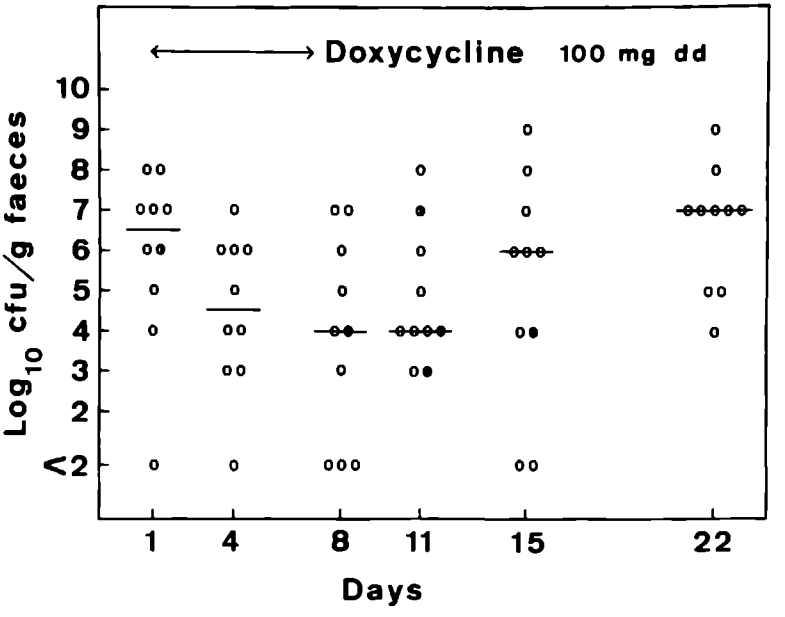

Figure 4. Faecal concentration of Gram-negative bacilli following doxycycline.
Legends as in Figure 4.

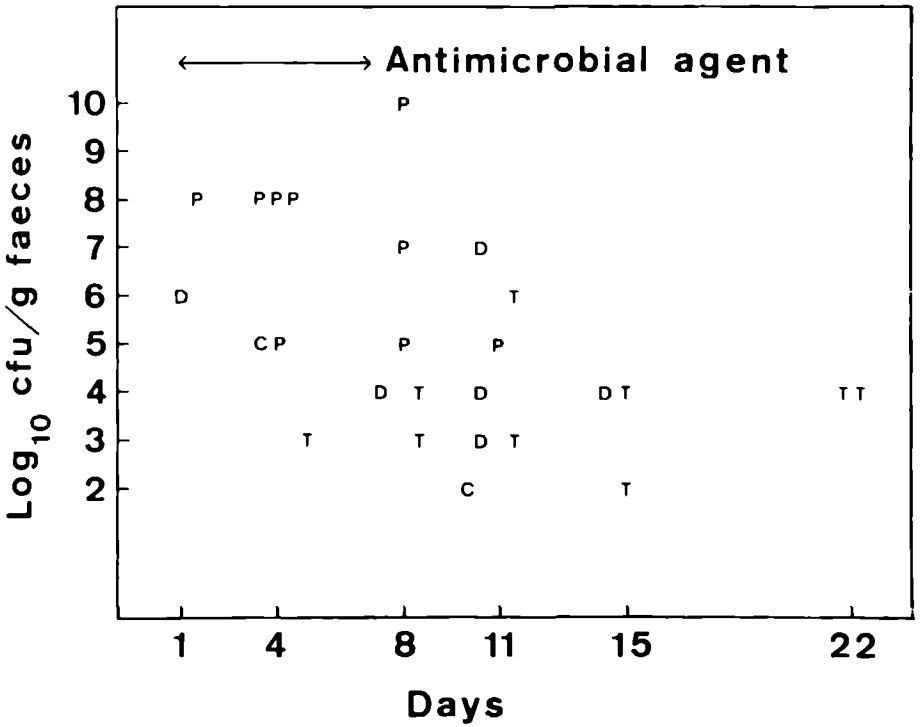

Figure 5. Faecal concentration of secondary Gram-negative bacilli following cefaclor (C), phenethicillin (P), co-trimoxazole (T), or doxycycline (D).

CHAPTER V

FAECAL LEVEL OF UROBILINOGEN: AN INDICATOR FOR THE RISK OF SUPERINFECTION AND OF FAILURE OF ORAL ANTICONCEPTION ?

E.J. Vollaard[a], M.M. Jankowiak[b], N.F. Muller[a] and H.A.L. Clasener[c]

Departments of [a]Pharmacy and [c]Microbiology, Canisius-Wilhelmina Hospital, St. Annastraat 289, 6525 GT Nijmegen, The Netherlands
[b]Department of Clinical Pharmacy, Medical Academy, Poznan, ul. Maria Magdaleny 14, PL 61861 Poznan, Poland

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Abstract

The influence of clindamycin, dicloxacillin, minocycline and norfloxacin on the faecal concentration of urobilinogen was investigated. The drugs were administered orally in standard dosage for six days to groups of six volunteers. A decrease in faecal concentration of urobilinogen following administration of clindamycin ($P < 0.01$) and dicloxacillin ($P < 0.05$) was found. The possible predictive value of a decrease of the faecal level of urobilinogen as an indicator for impairment of microbial colonization resistance (CR) and for the risk of failure of oral anticonceptive treatment is discussed. It is suggested that clindamycin and dicloxacillin should not be combined with oral anticonceptive treatment unless more specific investigations have excluded interaction of these drugs with oestrogen metabolism in the bowel.

Introduction

Disturbance of the microbial ecology of the gut is an important side effect of some antimicrobial agents. These antimicrobial agents eliminate a certain part of the anaerobic gut flora, which results in overgrowth of aerobic flora, thereby increasing the source and thus the risk of endogenous infections (1). As the part of the anaerobic flora that provides this so called colonization resistance (CR) against aerobic flora has not yet been identified, its presence has to be deduced from the limitation it causes of the concentration of the aerobic faecal flora. Elimination of the anaerobic CR flora by agents like amoxycillin, readily results in overgrowth by aerobic Gram-negative bacilli because resistant bacteria like *Klebsiella* are generally available (2). Elimination of the anaerobic CR flora may go unnoticed, however, as long as the patient harbours nor encounters micro-organisms resistant to the antimicrobial agent used. In experimental studies in animals or in human volunteers, aerobic bacteria resistant to the antimicrobial agent investigated may even have to be administered when no resistant aerobic flora is naturally present (challenge).
In studies in patients, another method is needed to assess the

presence of the CR flora. The faecal concentration of bèta-aspartylglycine has been used as a metabolic indicator for disturbance of CR. However, the bèta-aspartylglycine test is too insensitive (1). The report that some antimicrobial agents which are known to affect the CR flora, decreased the faecal concentration of urobilinogen, a product of bacterial metabolism (3), suggested the possibility of another method.

The concentration of urobilinogen in faeces might also be used as an indicator of the influence of antimicrobial agents on the capacity of the bowel flora to deconjugate glucuronides. Conjugated bilirubin is excreted by an active transport process from hepatic cells into the bile, mainly as glucuronides. In the large bowel it is deconjugated and converted to urobilinogen by intestinal micro-organisms. Suppression of the deconjugating bacterial flora will decrease the production and thus the level of urobilinogen in faeces. In germfree animals, for example, no urobilinogen is present in faeces (4).

Suppression of deconjugating bacterial flora in the bowel decreases enterohepatic cycling of oestrogens and therefore decreases the blood level of oestrogens. This might cause failure of oral anticonception (5). Therefore, the faecal level of urobilinogen might predict which antibiotics cause a risk for females on oral anticonceptive agents.

Investigators of the Karolinska Institute at Stockholm, Sweden, have described the influence of several antimicrobial agents on the faecal level of urobilinogen (3, 6). However they only gave data on the level of urobilinogen before treatment and on day six of treatment. In this pilot study the time course of the influence of clindamycin, dicloxacillin, minocycline and norfloxacin on urobilinogen production was investigated. The urobilinogen level was determined by a semi-quantitative solid-phase chemical method using Urobilistix (R) test strips.

Methods

Subjects

Twelve healthy adults (7 male and 5 female) aged between 22 and 35 years (median age 28) volunteered for this study. They had no

significant medical history, in particular no renal or liver disease, and no hypersensitivity to the studied drugs. Routine screening tests, performed before and after the study, consisted of a full blood count, urine analysis and liver function tests. The volunteers did not use any drug within 2 months before and during the investigation. Female volunteers were warned that use of antibiotics might result in failure of oral anticonception. Written informed consent was given prior to the start of the study, which was performed in accordance with the revised Declaration of Helsinki.
Volunteers were questioned about side effects after each study.

Study design
The volunteers were randomly divided into two groups. Group I (5 males and 1 female) received norfloxacin (Noroxin (R), Merck Sharp & Dohme, Haarlem, the Netherlands) tablets 400 mg twice daily for six days, and after a wash-out period of six weeks, minocycline (Minocin (R), Lederle Nederland BV, Etten-Leur, the Netherlands) capsules 100 mg twice daily for six days. Group II (2 males and 4 females) received dicloxacillin (Diclocil (R), Bristol-Meyers, Weesp, the Netherlands) capsules 500 mg four times a day for six days, and after a wash-out period of six weeks, two clindamycin (Dalacin C (R), Upjohn, Ede, the Netherlands) capsules 150 mg twice daily for six days.
Administration of the drugs started at day 0. The faecal sample 24 hours after the first antibiotic administration was indicated as the first treatment sample (day 1). The faecal sample of the first day after the termination of antibiotic treatment was considered as the last treatment sample. The sample of day 0 was not included in analysis.
From each volunteer daily pre-treatment samples were investigated, taken on five successive days before each of the two antibiotic periods.
Faecal samples were analyzed daily during the drug period and on days 1-5, 10 and 14 of the post treatment period.

Urobilinogen assay
The faecal level of urobilinogen was determined by a semi-quantitative solid-phase chemical method, using Urobilistix R

(Ames, Division Miles, (France) test strips. These test-strips employ the Ehrlich-reaction, which has been used before for determination of faecal urobilinogen by the Karolinska-group (4). The reaction is disturbed by the presence of nitrite. Nitrite-tests were negative however in 50 faecal samples in a pilot-study. Therefore, in the final study we omitted the nitrite-reaction.
Because of the decomposition of urobilinogen in time, all samples were analyzed immediately, or they were stored in a refrigerator and analyzed within 2 hours after delivery into sample containers. Of the faeces 0,3 - 0,7 g were mixed with 5 ml of 0,9% NaCl solution. The samples were vigorously shaken by Vortex, until homogeneous suspensions were obtained. After centrifugation at 1,000 g for 3 minutes, a test-strip was dipped in supernatant.
The faecal concentration of urobilinogen was calculated according to the formula:

$$C_{UB} = R \times \{(5.0 + W_F)/W_F\} \times 10^{-3} \text{ mmol/l}$$

where C_{UB} is the faecal concentration of urobilinogen, R is the result of test strip-reading (micromol/l), W_F is the weight of the faecal sample and 5.0 is the weight of 5 ml 0,9% NaCl solution.
The result is found in mmol/l faeces. As the specific gravity of faeces is about 1.0 final results will be expressed in mmol/kg faeces. All determinations were done in triplicate.

Statistical analysis:
Friedman two-way analysis by ranks was used to test the changes of urobilinogen level during the pre-treatment and the treatment period. Significance was assigned to a two tailed *P* value of less than 0.05.

Results

Faecal level of urobilinogen
Because of technical problems no determinations were made on day 10 after the administration of clindamycin.
The mean faecal urobilinogen concentrations in the pre-treatment period and following the administration of antibiotics are shown in the Figure. The mean levels and ranges of urobilinogen before, during and after administration of antibiotics are shown in the

Table.
The level of urobilinogen decreased in all volunteers, following administration of each drug. The decrease was significant following clindamycin ($P < 0.01$) and dicloxacillin ($P < 0.05$), but not following norfloxacin and minocycline administration.

Tolerance
No abnormal values were found for the routine screening tests performed before and after drug treatment. However in group II, two in four female volunteers acquired symptomatic *Candida* vaginitis when clindamycin was administered and one volunteer when dicloxacillin was administered. The diagnosis was confirmed by culture. The symptoms disappeared within 3 days following application of a miconazole vaginal ovula 1,200 mg. Some gastrointestinal discomfort occurred in 3 volunteers during the clindamycin period.

Discussion

During clindamycin, the level of urobilinogen dropped to a median value of 0.3 mmol/kg (Table). It has been shown that clindamycin disturbs the microbial CR against aerobic flora, causing at least a 100-fold increase in indigenous aerobic flora (7). So, even if we assume that conversion of bilirubin glucuronides in our volunteers was caused exclusively by the indigenous aerobic flora, this could only produce about 0.003 mmol urobilinogen/kg faeces in normal concentration. As the normal level of urobilinogen is about 1.0 mmol/kg (Table), the influence of aerobic flora on the level of urobilinogen can be neglected. Therefore, decrease of the faecal level of urobilinogen indicates suppression of anaerobic flora.
Anaerobic flora is the major determinant of microbial CR against potentially pathogenic aerobic flora (1). Therefore, reduction of the faecal concentration of urobilinogen might predict disturbance of microbial CR. In the study of the Karolinska team (3, 6), all drugs which are considered to impair CR (1) significantly decreased the concentration of urobilinogen. In the present study a significant decrease of urobilinogen was found for clindamycin ($P < 0.01$), which disturbs CR (7) and for dicloxacillin ($P < 0.05$),

which is suspected of disturbing CR (1). No significant influence was found for norfloxacin and minocycline. Quinolones are presumed not to impair CR. For minocycline no data about influence on CR are available, but it is probably comparable to doxycycline, which does not disturb CR (8, 9).
So, the data of this pilot study is compatible with the hypothesis that the influence of antibiotics on the faecal level of urobilinogen is an indicator of their influence on microbial CR. Further study's are required which investigate this hypothesis directly.
The decrease of the faecal level of urobilinogen following certain antibiotics also predicts disturbance of intra-intestinal deconjugation of other substances than bilirubinglucuronides. This may have consequences for enterohepatic cycling and therefore for the blood levels of some drugs. Adlercreutz found a thirty-fold increase in the faecal concentration of conjugated oestrogens and a decrease of 30% in the concentration of free oestrogens in 24-hour urine in pregnant females who used ampicillin (10). In this way, antibiotics which inhibit intestinal glucuronidase activity, like ampicillin and tetracycline, might cause failure of oral anticonception with oestrogens (5).
In the Netherlands, a warning against possible induction of failure of oral anticonceptive drugs is not included in the package insert of clindamycin and dicloxacillin. However, until proof of the contrary is provided, clindamycin and dicloxacillin should be considered dangerous for users of oral anticonceptive agents.
In female patients on oral anticonceptive agents who need antimicrobial treatment, antimicrobial agents which do not decrease the faecal concentration of urobilinogen are to preferred, as long as the influence of individual antimicrobial agents on the blood level of oestrogens is unknown.

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Table. Median value and range of faecal urobilinogen (mmol/kg) in six volunteers

Sample*	Volunteers 1-6		Volunteers 7-12	
	norfloxacin	minocycline	dicloxacillin	clindamycin
Tpt(5)	1.0(0.7-1.4)	1.0(0.7-1.4)	1.1(0.9-1.4)	1.0(0.5-1.1)
1	0.9(0.3-2.4)	1.0(0.2-1.6)	0.6(0.3-0.9)	0.4(0.2-0.9)
2	0.7(0.3-1.6)	0.8(0.3-1.3)	0.4(0.2-0.7)	0.5(0.2-0.9)
3	0.6(0.3-1.4)	0.9(0.5-1.5)	0.5(0.2-0.7)	0.4(0.2-0.8)
4	0.6(0.4-0.7)	0.8(0.5-1.1)	0.5(0.2-0.8)	0.3(0.0-0.4)
5	0.7(0.4-1.0)	0.8(0.5-1.4)	0.4(0.2-0.7)	0.3(0.0-0.4)
6	0.9(0.5-1.6)	0.7(0.4-0.9)	0.5(0.2-0.7)	0.3(0.1-0.4)
+1	0.8(0.5-1.6)	0.8(0.4-1.0)	0.5(0.3-0.8)	0.3(0.2-0.5)
+2	0.7(0.4-1,2)	0,8(0.3-1.3)	0.5(0.3-0.8)	0.3(0.2-0.4)
+3	0.8(0.4-1.2)	0.9(0.3-1.3)	0.5(0.3-0.8)	0.4(0.2-0.6)
+4	0.8(0.5-1.4)	0.8(0.4-1.2)	0.6(0.3-1.3)	0.5(0.3-1.0)
+5	1.1(0.6-1.9)	0.8(0.4-1.2)	0.6(0.3-1.5)	0.5(0.3-0.6)
+10	1.0(0.6-1.3)	0.9(0.5-1.1)	0.7(0.5-1.1)	ND
+14	1.0(0.7-1.5)	0.8(0.5-1.1)	0.8(0.6-1.0)	0.9(0.4-1.3)

*Tpt(5) : Median value of six volunteers of five pre-treatment days.
Samples 1 to +1 : Treatment samples (first sample after treatment is counted as last treatment sample).
Samples +2 to +14 : Post-treatment samples.
ND : not determined.

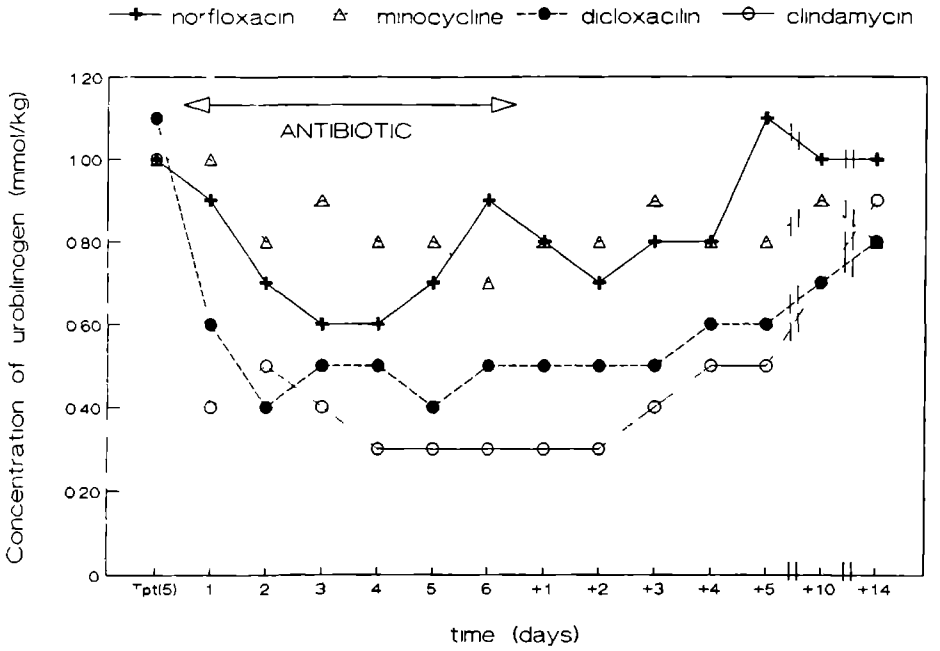

Figure. The influence of four antimicrobial agents on the faecal level of urobilinogen.
Samples Tpt(5) : Median value of six volunteers of five pre-treatment days.
Samples 1 - +1 : Treatment samples (first sample after treatment is counted as last treatment sample).
Samples +2 - +14 : Post-treatment samples.

CHAPTER VI

INFLUENCE OF AMOXYCILLIN ON MICROBIAL COLONIZATION RESISTANCE IN HEALTHY VOLUNTEERS. A METHODOLOGICAL STUDY

E.J. Vollaard[a], H.A.L. Clasener[b], A.J.H.M. Janssen[b] and H.J.A.Wynne[c]

Departments of [a]Pharmacy and [b]Microbiology, Canisius -Wilhelmina Hospital, St. Annastraat 289, 6525 GT Nijmegen, The Netherlands [c]Centre for Biostatistics, State University of Utrecht, Padualaan 14, 3584 CH Utrecht, The Netherlands

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Abstract

The influence of amoxycillin 500 mg tid on microbial colonization resistance (CR) was investigated in 11 healthy volunteers. Analysis was performed in each volunteer individually. In the first five volunteers we investigated the influence of amoxycillin on the faecal concentration of Gram-negative bacilli, enterococci and yeasts and on spontaneously occurring secondary colonization. In the next six volunteers we also investigated the influence of amoxycillin on CR against amoxycillin-resistant challenge strains, in order to be independent of the accidental presence of resistant Gram-negative bacilli.

In three volunteers all indicators employed did not show disturbance of the anaerobic flora that provides CR.

In five volunteers impairment of this flora was indicated both by increase of the faecal concentration of aerobic flora and by increase of spontaneously occurring secondary colonization or facilitation of colonization by the challenge strains. However, in the other three volunteers there was no concordance between the investigated indicators of the influence of amoxycillin on CR. Possible explanations are discussed. It is concluded that increase of the faecal concentration of aerobic flora is a more reliable indicator of impairment of the anaerobic flora that provides CR than increase of secondary colonization by strains acquired spontaneously or by challenge strains administered deliberately.

In one volunteer, who was excluded from the trial, high-level faecal colonization occurred after challenge with *Enterobacter cloacae* in the pre-treatment period.

Introduction

The autochthonous anaerobic flora limits the concentration of aerobic micro-organisms in the bowel ; this is known as microbial colonization resistance (1). The term colonization resistance was introduced by van der Waaij, to indicate the ability to withstand colonization of the bowel by aerobic challenge organisms (2). Subsequently the term colonization resistance has also been used to

indicate the limiting action of autochthonous anaerobic flora on the concentration of indigenous aerobic flora of the bowel (3).
The influence of antimicrobial agents on the anaerobic flora that provides CR cannot be investigated directly, because the composition of this flora is unknown (1). However, disturbance of this flora might be deduced from (a) increase of the concentration of aerobic flora, (b) increase of the concentration of secondary Gram-negative bacilli (Gram-negative bacilli other than *Escherichia coli*) (4), or (c) facilitation of colonization of the bowel by resistant challenge strains administered deliberately.
Until now, the correlation of these indicators of disturbance of the flora that provides CR has not been studied. Moreover, the influence of antimicrobial agents on CR in man has been investigated mostly by analyzing their influence on the median faecal concentration of aerobic flora in a group of volunteers. However, the concentration of antimicrobial agents in the bowel and the susceptibility to antibiotics of the anaerobic flora that provides CR may differ among volunteers. Therefore, this study was designed to investigate the influence of amoxycillin on CR in volunteers individually. Special attention has been given to the correlation of investigated indicators of impairment of CR in each volunteer.

Methods

Study design
In five volunteers (1-5) daily faecal samples were analyzed for their concentration of Gram-negative bacilli, enterococci and yeasts during a pre-treatment period of 20 days, a treatment period of 10 days and a post-treatment period of 10 days.
From the data derived from these volunteers it was calculated that a pre-treatment period and a treatment period of 10 days each would be sufficient to find a significant difference if the antimicrobial agent was able to cause a change in the concentration of aerobic flora of ten-fold or more. Therefore, in the next six volunteers the pre-treatment period was shortened to ten days (Volunteers 6-11). In these six volunteers we investigated the influence of amoxycillin on the faecal concentration of the aerobic flora as before. Moreover, to be independent of the accidental presence of resistant Gram-negative

bacilli, we also administered 10^6 cfu of amoxycillin-resistant challenge strains on the fifth day of the pre-treatment period and of the treatment period.
Daily faecal production was a selection criterion for the volunteers for this study. If more than one motion was passed, a sample of the morning portion was used for analysis. The data from the first day of antibiotic administration were not used for analysis. These samples cannot be used as pre-treatment samples as they may be influenced by drug treatment if they are produced after the first dosage of amoxycillin. On the other hand, the morning-samples of the first day of treatment will barely be influenced by the antibiotic. Therefore the faecal samples of the second day of treatment were counted as the first treatment samples and the morning-samples after the last day of amoxycillin-administration were counted as the last treatment samples.

Volunteers
Five healthy volunteers (two female, three male), age 27, 41, 42, 44 and 53, participated in the first part of the study. Six other healthy volunteers (three female, three male), age 23, 25, 32, 40, 48 and 51 participated in the second part of the study. The volunteers had not used antibiotics for at least one month before the start of the study. Written informed consent was obtained. Permission for this study was obtained from the ethics committee of the Canisius-Wilhelmina Hospital.

Drugs
Amoxycillin (Clamoxyl (R), Beecham) 500 mg capsules were administered three times daily after meals.

Challenge
As challenge strains we used a strain of *Klebsiella pneumoniae* and a strain of *Enterobacter cloacae*. Each strain was used in three volunteers of the second set (6-11). Both strains were highly resistant to amoxycillin (MICs 128 mg/l and 256 mg/l, respectively). The *K. pneumoniae* strain was highly resistant to pefloxacin (MIC = 56 mg/l) but susceptible to co-trimoxazole and cefotaxime. The *E. cloacae* strain was highly resistant to cefotaxime (MIC = 256 mg/l) but sensitive to quinolones. In this way, the challenge strains could

easily be isolated from faeces with selective culture media. At the same time, effective antibiotics would be available in case of infection by the challenge strains. The challenge strains were administered on the fifth day of the pre-treatment and of the treatment period in a dosage of 10^6 cfu. For administration, colonies of the challenge strain, grown overnight on solid media, were suspended in a glass of water, and drunk after a warm meal. In this situation, the bacterial suspension was expected to bypass the acidic environment of the stomach.

Bacteriology

Serial 1/10 dilutions of faeces were made in Thioglycollate medium (BBL). One-microlitre volumes of each dilution were inoculated on to solid media to isolate Gram-negative bacilli (Eosin methylene-blue lactose sucrose agar, Merck), amoxycillin-resistant Gram-negative bacilli (5% sheep blood in blood agar base, Oxoid, with amoxycillin 10 mg/l), cefotaxime-resistant Gram-negative bacilli (5% sheep blood in blood agar base, Oxoid, with cefotaxime 32 mg/l), pefloxacin-resistant Gram-negative bacilli (Eosin methylene-blue lactose sucrose agar, Merck, with pefloxacin 5 mg/l), enterococci (5% sheep blood in blood agar base, Oxoid, with nalidixic acid 50 mg/l), and yeasts (Sabouraud dextrose agar, Gibco, with chloramphenicol 125 mg/l). The solid media were also inoculated with 100-microlitre volumes of the first 1/10 dilutions of faeces, lowering the detection limit to 100 micro-organisms per gram of faeces. Concentrations were expressed as the logarithms to the base of 10 of the counts per gram of faeces, rounded up or down to whole numbers.

Micro-organisms isolated were identified by standard laboratory methods.

Statistics

The influence of amoxycillin on the faecal concentration of aerobic flora was evaluated for each volunteer individually, using single-case statistical techniques (5, 6). The absence of serial dependency of the data within each volunteer was verified by computing auto-correlations for time lags of one to four days for each series of data. For time lags of two to four days the correlation coefficients scattered around zero value, as expected under the null-hypothesis of

zero correlation. For a time lag of one day a slight auto-correlation ($r = 0.14$) did not disprove sufficiently the prerequisite independence of observations. Thus the Mann-Whitney-Wilcoxon test could be employed to evaluate the influence of amoxycillin on the concentration of Gram-negative bacilli, enterococci and yeasts in faeces. The counts in the pre-treatment samples were used as a baseline.

Results

Volunteers

Volunteer 8 acquired symptomatic *Candida*-vaginitis on day 7 of the amoxycillin-period, confirmed by culture. Complaints disappeared within 2 days following local application of a miconazole vaginal ovula (Daktarin R) 1200 mg. Amoxycillin-treatment was not discontinued. The other volunteers did not experience major complaints, although most of them reported some increase in defecation frequency.

One volunteer showed an unexpected response to the *E. cloacae* challenge strain. In this volunteer (male, 36 years old) the faecal concentration of *E. cloacea* was 10^{10} cfu/g on the second day after the challenge dose. Before the challenge dose the aerobic faecal flora was quite normal: Gram-negative bacilli 10^6-10^8 cfu/g, enterococci 10^3-10^5 cfu/g and yeast 10^3 cfu/g. He had not used antibiotics or any other drugs for several years and didn't have any health problem. Although this volunteer remained completely symptom-free, it was decided to take him out of the trial and to start decontamination with norfloxacin 400 mg twice daily. The challenge strain was below detection limit in faeces (10^2 cfu/g) following 4 days of treatment with norfloxacin. The faecal concentration of *E. cloacae* had been 10^8-10^{10} cfu/g (median 10^9 cfu/g) for seven days. This volunteer was replaced by another one in this trial.

Faecal concentration of aerobic flora

The median level and range of the faecal concentration of Gram-negative bacilli, enterococci and yeasts in volunteers 1-5 are shown in Table I.

From the fluctuation of the faecal concentration of aerobic flora in the 20-day pretreatment period, it was estimated that a 10-day

pretreatment period would have been sufficient in these volunteers to demonstrate a ten-fold increase or decrease of faecal concentration with statistical significance ($P < 0.05$). Therefore it was decided to shorten the pre-treatment period to ten days in the next six volunteers. For comparison with the data of volunteers 6-11, the data of volunteers 1-5 were recalculated, using the data of the last ten days of the 20-day pretreatment period as a baseline for the amoxycillin-period. These data and the data of volunteers 6-11 are shown in Table II. In eight of 11 volunteers a significant increase in the faecal concentration of Gram-negative bacilli and/or yeasts was observed.

Challenge strain

In four of six volunteers amoxycillin did not facilitate colonization of the bowel by the challenge strain (Table III).

In volunteer 7 the challenge strain re-appeared in faeces on day 2 of the amoxycillin-period, following four days with negative faecal samples. The faecal samples remained positive as long as amoxycillin was administered, but they were negative again after day 1 of the post-treatment period.

In volunteer 11 the first sample positive for the challenge strain in the amoxycillin-period occurred on the day after the challenge. In this volunteer the faecal samples of the post-treatment period remained positive during the amoxycillin-period and during the 10 post-treatment days.

Spontaneous secondary colonization with Gram-negative bacilli

Table IV shows the influence of amoxycillin on the faecal concentrations of Gram-negative bacilli other than *E. coli* (secondary colonization), excluding the challenge strain.

Following amoxycillin significant increase of spontaneous secondary colonization was found in seven of eight volunteers in whom disturbance of the anaerobic flora that provides CR was also indicated by increase of the faecal concentration of aerobic flora (Table V). No increase in secondary colonization was found in the three remaining volunteers, in whom the faecal concentration of Gram-negative bacilli and yeasts did not increase. In all volunteers *E. coli* was the dominant species of Gram-negative bacilli in all pretreatment samples. Apart from the challenge strains, no pefloxacin-resistant or cefotaxime-resistant Gram-negative bacilli were detected.

During the amoxycillin-period the concentration of secondary Gram-negative bacilli exceeded the concentration of *E. coli* in faecal samples of some volunteers, as in our previous study with amoxycillin (4).

Discussion

Analysis of data in volunteers separately

This study confirms the impairment of CR against Gram-negative bacilli and yeasts by amoxycillin that we found in a previous study (4). In that study however, we applied conventional group analysis. We now think it is preferable to analyze the data in volunteers individually, because important data are lost when group-analysis is applied in biomedical studies if the study-subjects do not behave homogeneously (5, 6, 7). For example, amoxycillin or ceftriaxone do not always impair CR, probably because some people harbour bèta-lactamase-producing anaerobic flora. (8, 9). If the anaerobic flora that provides CR is not susceptible and *E. coli* is susceptible to amoxycillin, the faecal concentration of Gram-negative bacilli may even decrease, as happened in volunteer 4. If this occurs in a substantial part of the volunteers the median faecal concentration of Gram-negative bacilli of the group of volunteers may remain unchanged, even if CR is impaired in several volunteers.

Faecal concentration of aerobic flora

In our view, the total concentrations of Gram-negative bacilli, enterococci and yeasts in the digestive tract are limited by autochthonous obligate anaerobic flora (1). If the anaerobic flora that provides CR is disturbed by an antimicrobial agent, an increase is to be expected in the faecal concentration of Gram-negative bacilli, enterococci and yeasts, unless resistant species are not available. Most enterococci are sensitive to amoxycillin. In this study, all volunteers had at least one negative faecal sample for enterococci in the amoxycillin-period (Table II) and the median faecal concentration of enterococci decreased at least ten-fold in eight of 11 volunteers. In volunteer 10 a decrease was not possible because only two pretreatment samples were positive for enterococci. However, administration of amoxycillin may cause selection of resistant

enterococci in the bowel. In volunteer 8 amoxycillin eliminated enterococci from the first four faecal samples, but the following samples were positive and the faecal concentration of enterococci increased to 10^9 cfu/g in the last treatment sample. In this volunteer, the faecal concentration of enterococci in the last six samples of the amoxycillin-period was significantly higher than in the pre-treatment period ($P = 0.02$), indicating impairment of CR against enterococci. We conclude that in general the faecal concentration of enterococci is not suitable as an indicator for impairment of CR by amoxycillin, because most available enterococci are too sensitive. In this regard, volunteer 8 was exceptional.

Good correlation was found between the influence of amoxycillin on the faecal concentrations of Gram-negative bacilli and of yeasts (Table V). In seven volunteers (1, 2, 3, 5, 6, 7 and 8) both were increased and in three volunteers (4, 9 and 10) both were not increased. Only in volunteer 11 the significant increase in the faecal concentration of yeasts was not accompanied by a significant increase in the concentration of Gram-negative bacilli. In this volunteer however, increase of the faecal concentration of Gram-negative bacilli and yeasts was very small, indicating minor impairment of anaerobic flora (Table II). Moreover, the fluctuation of the faecal concentration of Gram-negative bacilli in faeces of this volunteer was exceptionally high. Therefore in this volunteer the number of samples was too small to expect statistically significant results for a tenfold increase of the faecal concentration of Gram-negative bacilli.

The data in volunteer 8 support the hypothesis that disturbance of the flora that provides CR causes an increase in the faecal concentration of Gram-negative bacilli as well as of enterococci and of yeasts. The simultaneous increase of the concentration of all three groups of aerobic flora in volunteer 8 indicates that none of them limits the concentration of the other ones. This supports the hypothesis that the limiting action on the concentration of aerobic flora is provided by anaerobic flora.

Secondary colonization

Apart from an increase in the faecal concentration of resistant indigenous aerobic flora, impairment of anaerobic flora that provides CR may also increase secondary colonization by strains acquired spontaneously or by challenge strains administered on purpose.

In three volunteers (4, 9 and 10) amoxycillin did not cause an increase in the faecal concentration of Gram-negative bacilli or yeasts, indicating no disturbance of the anaerobic flora that provides CR. In these volunteers increase of secondary colonization by the challenge strains (volunteers 9 and 10) or by spontaneously occurring Gram-negative bacilli (volunteers 4, 9 and 10) did also not occur (Tables III, IV, V). So, in these three volunteers all indicators for impairment of CR we investigated were negative (Table V). In five volunteers (1, 2, 3, 7 and 11) increase in their faecal concentrations of Gram-negative bacilli and/or yeasts indicated disturbance of the anaerobic flora that provides CR (Table II). In volunteers 1, 2, and 3 this was associated with an increase in secondary colonization by spontaneously occurring Gram-negative bacilli (Table V). (In these volunteers challenge strains were not administered). In volunteers 7 and 11 increase in the concentration of aerobic flora was associated with an increase of secondary colonization by both spontaneously occurring and intentionally administered Gram-negative bacilli (Tables III, IV, V). So, in these five volunteers both the data for secondary colonization and the data for the faecal concentration of aerobic flora indicated disturbance of the anaerobic flora that provides CR (Table V). However, in the remaining three volunteers (5, 6, 8), increase in the faecal concentration of aerobic flora was not associated with an increase of spontaneously occurring secondary colonization (volunteer 5) or facilitation of colonization by the challenge strain (volunteers 6 and 8). In volunteer 5, we found no statistically significant increase of the concentration of spontaneously occurring Gram-negative bacilli (Table IV). However, the first three treatment samples in this volunteer contained much higher concentrations of secondary Gram-negative bacilli (10^6-10^8 cfu/g) than all pretreatment samples (Table IV). In the succeeding faecal samples the concentration of *E. coli* increased to high levels and secondary colonization could no longer be detected. However, this does not prove absence of secondary colonization, because it cannot be excluded that secondary colonization persisted in a concentration lower than the faecal concentration of *E. coli*. This points to a technical problem in studying spontaneously occurring secondary colonization. To exclude low level secondary colonization in volunteer 5 an appropriate selective medium should have been chosen after determination of the

antibiotic sensitivities of the strain of *E. coli* and the secondary strain that colonized this volunteer in the first few days of the amoxycillin-period, but this was not foreseen in the study design. Of course it is still possible that in volunteer 5 secondary colonization really disappeared, for example by competition with the increasing number of organisms of indigenous *E.coli*.

In volunteers 6 and 8, not a single sample was positive for the *K. pneumoniae* challenge strain during the amoxycillin-period (Table III), although in both volunteers amoxycillin caused colonization by high concentrations of spontaneously acquired secondary Gram-negative bacilli. The sensitivity profile of the challenge strain and the use of selective medium would have made the challenge strain detectable, even if outnumbered by other Gram-negative bacilli. We suggest that the challenge strain could not demonstrate impairment of CR in volunteers 6 and 8 because it lost the competition with other secondary Enterobacteriaceae or *E. coli* (Table V). Competition between Gram-negative bacilli has been demonstrated before. In streptomycin- pretreated mice and guinea pigs *E. coli* accelerated elimination of *Shigella flexneri* from faeces (10). Moreover, it has been shown in an *in vitro* model that from two microbial species that compete for the same ecological niche (mucosal receptors or food substrate) a competitive advantage exists for the first arriving species (11). So the success of a challenge strain, even in subjects with impaired CR, will depend on the time of administration, on the characteristics of the challenge strain and on the susceptibility of indigenous aerobic species and of species in food to the antimicrobial agent under investigation. Therefore, challenge strains are not always the most sensitive indicators for impairment of CR.

We conclude that disturbance of the anaerobic flora that provides CR, frequently causes an increase in colonization of the bowel by secondary Gram-negative bacilli, but not necessarily so (false negative results). Conversely, on present evidence, it cannot be excluded that indigenous Gram-negative bacilli are also involved in the prevention of low-level secondary colonization of the bowel by exogenous Gram-negative bacilli (4, 12), making it uncertain whether increase of secondary colonization really proves disturbance of the anaerobic flora that provides CR (false positive results). So, because secondary colonization might be a false negative indicator as

well as a false positive indicator of disturbance of the anaerobic flora that provides CR, increase in the faecal concentration of aerobic flora is a more reliable indicator of impairment of this flora than increase of secondary colonization. Van Saene *et al.* concluded from their study with amoxycillin that secondary colonization by resistant challenge strains is the most sensitive indicator of impairment of CR (13). However, they used only three faecal samples from the amoxycillin period. Such a study design allows detection of facilitation of secondary colonization, but is not suitable to detect a small increase in the total faecal concentration of groups of aerobic flora. So, part of the difference between their conclusion and ours may be explained by difference in study design.

The high-level faecal colonization with *E. cloacae* in volunteer 12 after challenge in the pretreatment period was totally unexpected. To our knowledge, successful high-level colonization of the bowel in healthy volunteers, following challenge with a low dosage of secondary Gram-negative bacilli, has never been described. This volunteer had been healthy all his life. His faecal flora before the challenge was quite normal. If this volunteer was so susceptible to secondary colonization, it is difficult to understand why this did not happen before with strains ingested with food. Exceptional colonizing properties of the challenge strain were not found in the three other volunteers who ingested this strain, even although one of those (volunteer 7) was very susceptible to secondary colonization following administration of amoxycillin (Table IV).

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Table I. Influence of amoxycillin on aerobic faecal flora*, in the first part of the study

Volunteer number	Gram-negative bacilli A	Gram-negative bacilli B	Enterococci A	Enterococci B	Yeasts A	Yeasts B
1	6(6-8)	7(6-9)$^{+}$	5(3-6)	2(<2-5)$^{--}$	<2(<2-2)	2(<2-4)$^{++}$
2	6(2-7)	7(4-8)$^{++}$	5(3-8)	3(<2-5)$^{--}$	<2(<2-2)	3(<2-4)$^{++}$
3	6(4-7)	8(6-9)$^{++}$	7(6-9)	5(<2-8)$^{--}$	<2(<2-2)	4(3-6)$^{++}$
4	6(4-7)	4(3-5)$^{--}$	5(4-6)	<2(<2-5)$^{--}$	<2(<2-<2)	<2(<2-2)^{ns}
5	2(<2-4)	8(5-9)$^{++}$	7(6-8)	3(<2-7)$^{--}$	2(<2-3)	4(3-4)$^{++}$

* : Log_{10} median faecal concentration (cfu/g) and range.
A : Pre-treatment period (n=20) ; B : Treatment period (n=10).
$^{++}$ or $^{--}$: P < 0.01 ; $^{+}$ or $^{-}$: P < 0.05 ; ^{ns} : P > 0.05, two sided significance of a difference in concentration between periods A and B

Table II. Influence of amoxycillin on aerobic faecal flora* in 11 volunteers

Volunteer number	Gram-negative bacilli A	Gram-negative bacilli B	Enterococci A	Enterococci B	Yeasts A	Yeasts B
1	6(6-8)	7(6-9)$^{++}$	5(3-6)	2(<2-5)$^{--}$	<2(<2- 2)	2(<2- 4)$^{+}$
2	4(2-7)	7(4-8)$^{+}$	5(3-8)	3(<2-5)$^{--}$	2(<2- 2)	3(<2- 4)$^{++}$
3	6(4-7)	8(6-9)$^{++}$	7(6-9)	5(<2-8)$^{-}$	2(<2- 2)	4(3- 6)$^{++}$
4	6(4-7)	4(3-5)$^{--}$	5(4-6)	<2(<2-5)$^{--}$	<2(<2-<2)	<2(<2- 2)^{ns}
5	3(<2-4)	8(5-9)$^{++}$	6(6-8)	3(<2-7)$^{--}$	2(<2- 3)	4(3- 4)$^{++}$
6	5(3-6)	8(6-9)$^{++}$	4(3-5)	2(<2-3)$^{--}$	2(<2- 4)	3(3- 4)$^{+}$
7	6(4-7)	8(6-8)$^{++}$	6(3-8)	5(<2-6)^{ns}	4(3- 5)	5(4- 5)$^{+}$
8	3(<2-4)	8(3-9)$^{++}$	4(2-5)	6(<2-9)^{ns}	3(<2- 4)	5(3- 7)$^{++}$
9	7(4-8)	7(5-9)^{ns}	4(<2-5)	4(<2-6)^{ns}	<2(<2- 2)	<2(<2-<2)^{ns}
10	5(<2-7)	6(2-9)^{ns}	<2(<2-3)	<2(<2-3)^{ns}	3(<2- 4)	3(<2- 5)^{ns}
11	6(3-8)	7(3-8)^{ns}	5(4-6)	3(<2-4)^{ns}	3(2- 3)	3(3- 4)$^{+}$

* : Log_{10} median faecal concentration (cfu/g) and range.
A : Pre-treatment period (n=10) ; B : Treatment period (n=10).
$^{++}$ or $^{--}$: P < 0.01 ; $^{+}$ or $^{-}$: P < 0.05 ; ^{ns} : P > 0.05 , two sided significance of a difference between periods A and B.

Table III. Influence of amoxycillin on colonization by challenge strains

Volunteer number	Challenge strain[a]	Pre-treatment period C_{max}[b]	Pre-treatment period duration[c]	Treatment period C_{max}[b]	Treatment period duration[c]
6	K. pneumoniae	10^3	2	$<10^2$	0
7	E. cloacae	10^5	2	10^6	9[d]
8	K. pneumoniae	10^2	1	$<10^2$	0
9	E. cloacae	$<10^2$	0	$<10^2$	0
10	K. pneumoniae	$<10^2$	0	$<10^2$	0
11	E. cloacae	$<10^2$	0	10^6	>15[e]

[a] : Oral administration of 10^6 cfu on fifth day of pre-treatment and treatment period. [b] : Maximal faecal concentration (cfu/g). [c] : Number of days with positive faecal samples. [d] : Challenge strain re-appeared spontaneously on day 2 of amoxycillin period. [e] : Challenge strain had not disappeared at day 10 of the post-treatment period.

Table IV. Influence of amoxycillin on faecal concentration[a] of Enterobacteriaceae other than E. coli (secondary colonization)[b]

Volunteer number	Pre-treatment period (n=10)	Treatment period (n=10)
1	<2 (<2- 4)	3 (<2- 6)$^{+}$
2	<2 (<2- 6)	5 (4- 7)$^{++}$
3	3 (<2- 6)	7 (3- 8)$^{++}$
4	<2 (<2-<2)	<2 (<2-<2)^{ns}
5	2 (<2- 3)	<2 (<2- 8)^{ns}
6	<2 (<2-<2)	8 (<2- 9)$^{++}$
7	2 (<2- 4)	7 (<2- 8)$^{++}$
8	<2 (<2- 3)	8 (2- 9)$^{++}$
9	<2 (<2-<2)	<2 (<2-<2)^{ns}
10	3 (<2- 5)	2 (<2- 8)^{ns}
11	<2 (<2-<2)	3 (<2- 5)$^{++}$

[a] : Log_{10} median concentration (cfu/g) and range. [b] : Challenge strains are not taken into account.
$^{++}$ or $^{--}$: $P < 0.01$; $^{+}$ or $^{-}$: $P < 0.05$; ^{ns} : $P > 0.05$, two sided significance of a difference in concentration between periods A and B.

Table V. Influence of amoxycillin on indicators of impairment of colonization resistance of the bowel

Volunteer number	Faecal concentration of: Gram negative bacilli	Yeasts	Secondary GNB[a]	Challenge Strain
1	+	+	+	#
2	+	+	+	#
3	+	+	+	#
4	-	-	-	#
5	+	+	-	#
6	+	+	+	-
7	+	+	+	+
8	+	+	+	-
9	-	-	-	-
10	-	-	-	-
11	-	+	+	+

[a] : Gram-negative bacilli.
: Not done.
+ : Increase.
- : No increase.

CHAPTER VII

INFLUENCE OF CEFOTAXIME ON MICROBIAL COLONIZATION RESISTANCE IN HEALTHY VOLUNTEERS

E.J. Vollaard[a], H.A.L. Clasener[b], A.J.H.M. Janssen[b] and H.J.A. Wynne[c]

Departments of [a]Pharmacy and [b]Microbiology, Canisius-Wilhelmina Hospital, St. Annastraat 289, 6525 GT Nijmegen, The Netherlands [c]Centre for Biostatistics, State University of Utrecht, Padualaan 14, 3584 CH Utrecht, the Netherlands.

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Abstract

The influence of cefotaxime 1000 mg given intravenously bid on colonization resistance (CR) was investigated in six healthy volunteers. Administration of cefotaxime allowed colonization of the bowel by a resistant challenge strain of *Enterobacter cloacae* in all volunteers. The faecal concentration of aerobic flora increased significantly in five of six volunteers. In one the faecal concentration of Gram-negative bacilli, enterococci and yeasts increased. In the other four the faecal concentration of enterococci and yeasts increased, but Gram-negative bacilli did not rise above pretreatment level. It is concluded that cefotaxime impairs CR, although to a variable degree. Therefore the term "selective decontamination" is not fully justified for prophylactic regimens that include cefotaxime.

Introduction

Microbial CR limits the concentration of indigenous aerobic flora of the digestive tract and its colonization by potentially pathogenic aerobic micro-organisms ingested with food. Microbial CR is probably provided by autochthonous anaerobic flora (1,2). Therefore it should be possible to eliminate aerobic flora from the bowel without impairing CR. This is called selective decontamination. Prophylactic regimens designed to provide selective decontamination have been used successfully in neutropenic patients (1), in patients on mechanical ventilation (3), and in patients with transurethral catheters (4). However, selectivity to aerobic flora has not been proved for any of these regimens by challenge with resistant micro-organisms. Therefore, it is possible that the success of these regimens is caused by their broad spectrum of activity against aerobic flora and high concentration in the digestive tract, despite some disturbance of the anaerobic flora that provides CR.

Parenteral administration of cefotaxime is included in most regimens for selective decontamination in intensive care patients, to prevent early infection with indigenous flora (5). Cefotaxime was chosen as part of the original Groningen-regimen, because of its useful spectrum against pulmonary pathogens and the absence of influence on microbial CR in an unpublished mouse-study (3). However, in a

recent study it was found that cefotaxime impaired CR against enterococci in mice treated according to the Groningen-regimen (6). Moreover, the influence of cefotaxime on microbial CR has never been investigated in man. We therefore decided to investigate the influence of cefotaxime on microbial CR in human volunteers. Because we expected a low prevalence of cefotaxime-resistant Gram-negative bacilli in the bowel of our volunteers and in food, we administered challenge doses of a highly resistant strain of *Enterobacter cloacae*.

Methods

Study design
Daily faecal samples from volunteers were analyzed for the concentration of Gram-negative bacilli, enterococci and yeasts during a pre-treatment period, a cefotaxime-period and a post-treatment period of ten days each. If more than one motion was passed, a sample of the first motion of that day was used for analysis. The faecal samples of the first day of the cefotaxime-period were not used for analysis and the sample of the first day after the cefotaxime-period was counted as the last treatment sample, as described before (7). On the third day of the pretreatment period , the normal CR of the volunteers was challenged by oral administration of a cefotaxime-resistant strain. The challenge was repeated on the third day of the cefotaxime period.

Volunteers
Six male healthy volunteers, aged 19-44 years, participated in the study. The volunteers had not used antibiotics for at least one month prior to the start of the study.
Written informed consent was obtained and permission for this study was obtained from the ethics committee of the Canisius-Wilhelmina hospital.

Drugs
Cefotaxime 1000 mg was administered intravenously twice daily at 9.00 and at 17.00 hours. The antibiotic was dissolved in 4 ml water for injection and slowly injected.

Challenge
As a challenge strain we administered 10^6 cfu of a strain of *Enterobacter cloacae* , highly resistant to cefotaxime (MIC = 256 mg/l), but sensitive to quinolones. In this way, the challenge strain could easily be isolated with selective culture medium. At the same time, effective treatment would be available in case of infection with the challenge strain. The same strain has been used in a previous study with amoxycillin (7).

Bacteriology
Serial 1/10 dilutions of faeces were made in Thioglycollate medium (BBL). One-microlitre volumes of each dilution were inoculated on to solid media to isolate Gram-negative bacilli (Eosin methylene-blue lactose sucrose agar, Merck), the challenge strain (Mc Conkey agar with cefotaxime 32 mg/l), amoxycillin-resistant Gram-negative bacilli (5% sheep blood in blood agar base, Oxoid, with amoxycillin 10 mg/l), enterococci (5% sheep blood in blood agar base, Oxoid, with nalidixic acid 50 mg/l), and yeasts (Sabouraud dextrose agar, Gibco, with chloramphenicol 125 mg/l). The solid media were also inoculated with 100-microlitre volumes of the first 1/10 dilutions of faeces, lowering the detection limit to 100 micro-organisms per gram of faeces. Concentrations were expressed as the logarithms to the base of 10 of the counts per gram of faeces, rounded up or down to whole numbers.
Micro-organisms isolated were identified by standard laboratory methods.

Statistics.
Because the faecal concentration of antibiotics and the antibiotic-sensitivity of the anaerobic flora that provides CR may differ between volunteers, the data were analyzed for each volunteer individually, using single-case statistical techniques (7). The absence of serial dependency of the data was verified by computing auto-correlations for time lags of one to four days for each series of data. For time lags of two to four days the correlation coefficients scattered around zero value, as expected under the null-hypothesis of zero correlation. For a time lag of one day a slight autocorrelation (r = 0.15) did not disprove sufficiently the prerequisite independence of observations. Thus the Mann-Whitney-Wilcoxon test could be

employed to evaluate the influence of cefotaxime on the faecal concentrations of Gram-negative bacilli, enterococci and yeasts. The counts in the pretreatment samples were used as a baseline.

Results

Volunteers

Volunteer 1 got bowel cramps on the sixth day and bloody diarrhoea on the seventh day of cefotaxime-administration. The faecal concentration of *E. cloacae* in the sample of the first day after the challenge was 10^{10} cfu/g. Therefore oral administration of norfloxacin 400 mg bid was started and administration of cefotaxime was stopped. Because *Clostridium difficile* toxin was also found the volunteer was treated with oral metronidazole 500 mg tid at the same time. Three days later *E. cloacae* and *C. difficile* toxin were absent. Complaints gradually subsided and disappeared in about five days. No problems occurred in the other volunteers.

Faecal concentration of aerobic flora (Table I).

The faecal concentration of *Escherichia coli* declined in all volunteers following administration of cefotaxime. After the challenge, the concentration of *E. coli* could not be determined in four of six volunteers, because it was lower than the concentration of the challenge strain and because a selective medium was not available for isolation of *E. coli* when outnumbered by the challenge strain.

In volunteer 1 the faecal concentration of *E. cloacae* in the four samples between challenge and start of norfloxacin-therapy was significantly above the faecal concentration of Gram-negative bacilli in the pre-treatment period ($P < 0.01$). In the other five volunteers cefotaxime did not cause an increase in the faecal concentration of Gram-negative bacilli, because the concentration of *E. cloacae* did not increase above the original concentration of *E. coli*.

The median faecal concentration of enterococci increased 100- to 10,000-fold in five of six volunteers. The median faecal concentration of yeasts increased ten-fold in the same volunteers.

Challenge strain (Table II).
In the pre-treatment period, the faecal concentration of the challenge strain remained below detection limit (10^2 cfu/g) in three of six volunteers. In the other volunteers the challenge strain could be detected for one, three and four days respectively.
Cefotaxime markedly impaired resistance against colonization by the challenge strain. Volunteer 1 had one morning-sample with 10^{10} cfu/gram and three morning-samples with 10^8 cfu/g of *E. cloacae*, before he was taken out of the trial because of side effects, as previously described. In the other volunteers the bowel remained colonized with *E. cloacae* as long as cefotaxime was administered. In volunteers 4 and 5, post-treatment samples remained positive for three and five days respectively. In volunteers 2, 3 and 6 all ten post-treatment samples remained positive.

Spontaneous secondary colonization with Gram-negative bacilli.
In the pre-treatment period, faecal colonization with Gram-negative bacilli other than *E. coli* (secondary colonization with Gram-negative bacilli) was not detected in volunteers 2, 3 and 4. In volunteers 1 and 5 only one sample was positive. In volunteer 6, six of ten samples contained *Acinetobacter anitratus*. In the cefotaxime-period only *E. coli* and the challenge strain were observed in volunteers 1, 2, 3, 4 and 5. In volunteer 6 the *A. anitratus* persisted up to the challenge with *E. cloacae*. *E. cloacae* was the dominant species of Gram-negative bacilli in the succeeding samples.

Discussion

It is assumed that the concentration of aerobic flora in the digestive tract is limited by autochthonous obligate anaerobic flora, and that disturbance of this flora by administration of an antimicrobial agent will cause an increase of aerobic flora if resistant species are present (1, 2). Since the anaerobic flora that provides this CR is unknown, disturbance of this flora can only be measured indirectly (7).
The aerobic faecal flora might be divided into three groups : Gram-negative bacilli, Gram-positive cocci and yeasts. To our knowledge it has never been shown that one of these groups limits the faecal concentration of one of the other two.

If there is one anaerobic flora that provides CR, disturbance of this flora will cause a simultaneous increase of the faecal concentration of all three groups of aerobic flora. In a previous study with amoxycillin we found no impairment of CR in three of 11 volunteers. In seven of eight of the other volunteers the faecal concentrations of both Gram-negative bacilli and yeasts were significantly increased. In these volunteers the faecal concentration of enterococci was not increased, but this could be explained by a low prevalence of amoxycillin-resistant enterococci (7).

The influence of cefotaxime on indicators of disturbance of the CR flora is less clear-cut (Table III). In volunteer 6 the faecal concentrations of Gram-negative bacilli, enterococci and yeasts were not increased, indicating no disturbance of the flora that provides CR. In volunteer 1 the faecal concentrations of all three groups of aerobic flora were increased, indicating disturbance of the flora that provides CR. In volunteers 2, 3, 4 and 5 however, the faecal concentrations of enterococci and yeasts were increased, but the concentration of Gram-negative bacilli did not increase above pre-treatment level, although these volunteers were colonized with the resistant challenge strain. An explanation might be that the concentration of Gram-negative bacilli in the bowel is limited by other anaerobic species than the concentration of yeasts and of aerobic Gram-positive cocci. Further research is required to test this theory.

Cefotaxime caused colonization by the challenge strain in all six volunteers. However, in volunteer 6 the faecal concentration of Gram-negative bacilli, enterococci and yeasts did not increase above pretreatment level. So, in this volunteer, facilitation of secondary colonization with the resistant challenge strain appeared to be the most sensitive indicator of impairment of the flora that provides CR against Gram-negative bacilli. Conversely, increase of the faecal concentration of Gram-negative bacilli was the most sensitive indicator of disturbance of CR-flora in a previous study with amoxycillin (7). In that study, however, the challenge strain had to compete with increased concentrations of resistant Gram-negative bacilli present before the challenge, while in this study with cefotaxime the concentration of indigenous Gram-negative bacilli was decreased.

Cefotaxime did not increase secondary colonization by spontaneously occurring Gram-negative bacilli, confirming a low prevalence of cefotaxime-resistant strains in the bowel of volunteers and in food.

The incidence of superinfections following cefotaxime has been reported to be remarkably low (8). Nevertheless, our study shows that cefotaxime 1000 mg intravenously bid impairs microbial CR in human volunteers. Therefore, the same can be expected in patients. The increase of the faecal concentration of enterococci following administration of third generation cephalosporins might be one explanation of the recent observation of a striking increase of the relative percentage of enterococcal infections in hospitals in the last decade {although the absolute percentage of these infections remains low ;(9)}. Increase of the faecal concentration of yeasts and enterococci in volunteers 1 - 5, and of *E. cloaceae* in volunteers 1-6 suggest that cefotaxime may increase the risk of infection by yeasts, by enterococci and by resistant Gram-negative bacilli. This is in agreement with the report that the 1.1% superinfections in 2,187 patients following cefotaxime were caused by *Pseudomonas* spp. (11 isolates), *Enterobacter* spp. (five isolates), *Candida* spp. (three isolates) and enterococci (two isolates), (10). In our study, spontaneous secondary colonization by Gram-negative bacilli other than the resistant challenge strain was not found in any volunteer during the cefotaxime-period. This confirms that the prevalence of cefotaxime-resistant Gram-negative bacilli is low. Moreover, the faecal concentration of the challenge strain remained low in five of six volunteers. On the contrary, following amoxycillin many volunteers are colonized by spontaneously occurring secondary Gram-negative bacilli in concentrations exceeding the original concentration of *E. coli* (7, 11). We therefore consider cefotaxime a safer drug than amoxycillin, concerning the risk of superinfections with Gram-negative bacilli; at least in a dosage of 2 gram daily and in case of normal kidney and liver function. The success of cefotaxime-containing prophylactic regimens (5) is achieved, however, **despite** some impairment of CR. Apparently, the antimicrobial spectrum of activity of these regimens is broad enough to prevent an increase in potentially pathogenic flora in the digestive tract.

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Table I. Influence of cefotaxime on faecal concentration of aerobic flora*

Volunteer number	Gram-negative bacilli A	Gram-negative bacilli B	Enterococci A	Enterococci B	Yeasts A	Yeasts B
1	7 (5-8)	(a) $^{++}$	5 (4-7)	8 (5-8)$^{++}$	2 (2-3)	3 (3-4)$^{+}$
2	7 (5-8)	6 (4-7)$^{--}$	4(<2-7)	6 (4-6)$^{++}$	2(<2-3)	3(<2-4)$^{+}$
3	7 (4-7)	6 (4-6)^{ns}	4 (3-7)	8 (5-8)$^{++}$	2(<2-3)	3(<2-6)$^{++}$
4	4(<2-6)	4(<2-5)^{ns}	3(<2-4)	6 (3-7)$^{++}$	2(<2-3)	3 (2-3)$^{++}$
5	6 (5-8)	5 (3-6)^{ns}	3(<2-6)	7(<2-7)$^{++}$	2(<2-2)	3 (2-4)$^{++}$
6	6(<2-6)	5(<2-6)^{ns}	4(<2-5)	4(<2-6)^{ns}	3(<2-4)	3(<2-4)^{ns}

* : Log_{10} median faecal concentration (cfu/g) and range.
A : Pre-treatment period (n=10); B : Treatment period (n=10, except for volunteer 1; n=6).
(a) : Significant increase ($P < 0.01$) for four samples between challenge and termination of treatment because of side effects.
$^{++}$ or $^{--}$: $P < 0.01$; $^{+}$ or $^{-}$: $P < 0.05$; ^{ns} : $P > 0.05$, two sided significance of a difference in concentration between periods A and B.

Table II. Influence of cefotaxime on colonization of the bowel by the challenge strain[a]

Volunteer number	Pretreatment Period Cmax[b]	Pretreatment Period duration[c]	Treatment Period Cmax	Treatment Period duration	Post-treatment Period duration
1	$<10^2$	0	10^{10}	6[d]	#
2	10^2	3	10^6	8	10
3	$<10^2$	0	10^6	8	10
4	$<10^2$	0	10^5	8	3
5	10^3	1	10	8	5
6	10^5	4	10^6	8	10

[a] : 10^6 cfu Enterobacter cloacae ($MIC_{cefotaxime}$ = 256 mg/l) on the third day of pre-treatment and treatment period.
[b] : Maximal faecal concentration (cfu/g).
[c] : Number of days with positive faecal samples.
[d] : Cefotaxime administration terminated on this day because of side effects.
: Administration of cefotaxime stopped in treatment period.

Table III. Influence of cefotaxime on indicators of impairment of colonization resistance of the bowel.

Volunteer number	Faecal concentration of: Gram-negative bacilli	Enterococci	Yeasts	Challenge strain
1	+	+	+	+
2	-	+	+	+
3	-	+	+	+
4	-	+	+	+
5	-	+	+	+
6	-	-	-	+

+ : Increase.
- : No increase.

CHAPTER VIII

INFLUENCE OF CEPHRADINE ON MICROBIAL COLONIZATION RESISTANCE IN HEALTHY VOLUNTEERS

E.J. Vollaard[a], H.A.L. Clasener[b] and A.J.H.M. Janssen[b].

Departments of [a]Pharmacy and [b]Microbiology, Canisius-Wilhelmina Hospital, St. Annastraat 289, 6525 GT Nijmegen, The Netherlands

Abstract

The influence of cephradine, 500 mg twice daily, on microbial colonization resistance (CR) was investigated in six healthy volunteers. Increase in the faecal concentration of Gram-negative bacilli, aerobic Gram-positive cocci or yeasts and increase in secondary colonization by strains acquired spontaneously or administered deliberately (challenge), were used as indicators for disturbance of the flora that provides CR. In two of six volunteers none of these indicators were found to be positive. In the other four volunteers minor impairment of CR was found. It is concluded that the flora that provides CR is not spared completely in all persons following cephradine 1 gram daily. However, the degree of impairment is much less than following amoxycillin. Therefore, the risk of superinfections is probably lower following cephradine.

Introduction

Antimicrobial agents may cause superinfections by disturbing the autochthonous anaerobic flora that provides CR (1). Therefore, it is preferable to select antibiotics for prophylaxis and for therapy that do not impair CR (2). Impairment of CR may go unnoticed if the prevalence of Gram-negative bacilli resistant to the intestinal concentration of the antibiotic is low, and if the number of samples in the study is too low to detect a small increase in the faecal concentration of indigenous aerobic flora (3). Therefore, up to now not a single antibiotic has been reliably classified as not sparing the microbial CR.

At the first international congress on CR, it has been suggested that cephradine does not impair CR up to a dosage of 9 gram daily (4). Afterwards however, it has been found that cephradine strongly impairs CR in almost half of the patients in a dosage of 6 g daily (1). At a dosage of 1.5 g daily, cephradine selected much less resistant Gram-negative bacilli in faeces than amoxycillin 1.5 g daily (5). This suggests that cephradine at this dosage has less influence on CR than amoxycillin, but the difference could also be caused by a lower prevalence of cephradine-resistant strains than of amoxycillin-resistant strains.

Therefore we decided to start a new study for investigation of the influence of cephradine on the microbial CR, using a sufficiently high number of samples to detect a small increase in the faecal concentration of yeasts (3), and including challenge with a cephradine-resistant challenge strain.
We used a dosage of 500 mg twice daily because we consider this dosage sufficiently high for treatment of urinary tract infections.

Methods

Study design
Daily faecal samples were analyzed for their concentrations of Gram-negative bacilli, aerobic Gram-positive cocci and yeasts during a pre-treatment period, a treatment period and a post-treatment period of ten days each. 10^9 cfu of a cephradine-resistant challenge strain was administered at day 2 of the pre-treatment period and at day 5 of the treatment period.

Volunteers
Six healthy volunteers, one female (age 52 years) and five male (age 20 - 49 years ; median age 41 years) participated in the study. The volunteers had not used antibiotics for at least one month before the start of the study. Written informed consent was obtained from the volunteers and permission for the study was granted by the ethics committee of the Canisius-Wilhelmina Hospital.

Drug
Cephradine 500 mg capsules were administered twice daily after meals.

Challenge
As a challenge, we used a strain of *Klebsiella pneumoniae*, that has been described before (3). The MIC for cephradine is 32 mg/l and the MIC for pefloxacin is 56 mg/l. Therefore, this strain can easily be isolated from faeces with selective culture medium. Because this strain is highly sensitive to cefotaxime and to co-trimoxazole, effective antibiotics are available in case of infection with the challenge strain.

An oral dose of 10^9 cfu of the challenge strain was administered on the second day of the pretreatment period and on the fifth day of the treatment period. Colonies of the challenge strain grown overnight on solid medium were suspended in saline. This suspension was poured in a glass of water, and drunk after a warm meal, immediately followed by another glass of water. In this way, the bacterial suspension was expected to bypass the acidic environment of the stomach.

Bacteriology

Serial 1/10 dilutions of faeces were made in Thioglycollate medium (BBL). One microliter-volumes of each dilution were inoculated on to solid media to isolate Gram-negative bacilli (eosin-methylene-blue lactose sucrose agar, Merck), amoxycillin-resistant Gram-negative bacilli (5% sheep-blood in blood agar base, Oxoid, with amoxycillin 10 mg/l), pefloxacin-resistant Gram-negative bacilli (McConkey agar, Oxoid, with norfloxacin 8 mg/l), enterococci and streptococci (5% sheep blood in blood agar base, Oxoid, with nalidixic acid 50 mg/l), staphylococci (mannitol salt agar, bió Merieux), and yeasts (Sabouraud dextrose agar, Gibco, with chloramphenicol 125 mg/l). The solid media were also inoculated with 100-microlitre volumes of the first 1/10 dilutions of faeces, lowering the detection limit to 100 micro-organisms per gram of faeces. The detection limit of the challenge strain was lowered to 10 micro-organisms per gram of faeces by inoculating 1 ml-volumes of the first 1/10 dilution of faeces on to extra-large Petri-dishes (diameter 14 cm) containing the specific medium. Concentrations of micro-organisms were expressed as the logarithms to the base of 10 of the counts per gram of faeces, rounded up or down to whole numbers. Micro-organisms isolated were identified by standard laboratory methods.

Faecal concentration of cephradine

Faecal concentration of cephradine was determined by an agar-diffusion method. Faeces was put in a well in a solid medium (isosensitest-agar, Oxoid), seeded with *Staphylococcus aureus* (ATCC 29213). The MIC of this strain for cephradine is 1 mg/l. The diameter of the inhibition zone was compared to the diameters of the inhibition zones around standard solutions of cephradine in

normal saline. The concentration found in this way will be called the diffusible faecal concentration of cephradine

Statistics

The influence of cephradine on the faecal concentrations of aerobic micro-organisms in faeces was evaluated for each volunteer individually, using single case statistics as described before (3). The absence of serial dependency of the data within volunteers was verified by computing auto-correlations for time lags of one to four days for each series of data. The correlation coefficients scattered around zero value, as expected under the null-hypothesis of zero correlation. Thus the Mann-Whitney-Wilcoxon test could be employed to evaluate the influence of cephradine on the concentrations of aerobic flora in faeces. The counts in the pre-treatment samples were used as a baseline.

Results

The diffusible faecal concentration of cephradine was below detection limit (8 mg/l) in all samples.

The influence of cephradine on the faecal concentration of aerobic flora was small (Tables I-IV). The total faecal concentration of Gram-negative bacilli increased significantly in volunteer 5 only, but the actual increase was very low.

In the pre-treatment period, *E. coli* was the dominant species of Gram-negative bacilli in all samples in all volunteers. Cephradine caused a strong increase in spontaneously occurring secondary colonization with Gram-negative bacilli in volunteers 3 and 5 (Table II). In both of these patients nine of ten treatment samples contained *Citrobacter freundii* (MIC for cephradine > 512 mg/l in both cases). *Citrobacter* remained detectable in the first eight samples of the post-treatment period in volunteer 3 and in all ten samples in volunteer 5. Colonization with the challenge strain was facilitated only in volunteer 6 (Table III).

Enterococci were the dominant species of aerobic Gram-positive cocci in almost all samples. Non-haemolytic staphylococci were dominant in three out of 30 samples in volunteer 3 and in one sample in volunteer 4. Streptococci were detected in all pre-

treatment samples in volunteer 3 (haemolytic streptococci group G), and in volunteer 5 (haemolytic streptococci group C) in a concentration about equal to the enterococci in both volunteers. Cephradine immediately eliminated the streptococci from faeces in both volunteers, and streptococci did not re-appear during the post-treatment period. The decrease in the faecal concentration of Gram-positive cocci in volunteer 5 was due to elimination of streptococci, the concentration of enterococci remained unchanged.
Cephradine caused a small, but significant increase in the faecal concentration of yeasts in three out of six volunteers (Table I).

Discussion

The autochthonous anaerobic flora that provides CR can not be measured directly. However, disturbance of this flora is indicated by an increase in the intestinal concentration of 1) Gram-negative bacilli, 2) aerobic Gram-positive cocci or 3) yeasts, or by an increase in the secondary colonization by strains 4) ingested with food or 5) administered deliberately (3). In volunteers 1 and 4 none of these five indicators of impairment of CR were found to be positive (Table IV). In the other four volunteers one or more indicators demonstrated disturbance of the anaerobic flora that provides CR. The concentration of yeasts was slightly but significantly increased in volunteers 2, 5 and 6, and the same applied to the concentration of Gram-negative bacilli in volunteer 5. In volunteers 3 and 5 a marked increase in secondary colonization was observed. So, although cephradine causes selection of Gram-negative bacilli less frequently than amoxycillin (5), cephradine is not devoid of the risk of this unfavourable effect.
Facilitation of colonization by the challenge strain administered deliberately was observed only in volunteer 6, but even in this volunteer the concentration of the challenge-strain declined during cephradine treatment and the strain was no more detected during the post-treatment period. The lack of facilitation of colonization by the challenge strain in volunteers 3 and 5, in whom susceptibility to secondary colonization appeared to be strongly increased, demonstrates again that the behaviour of a resistant challenge strain is not the most reliable indicator of the influence of antibiotics on

the flora that provide CR. It appears that the challenge strain may loose the competition with indigenous Gram-negative bacilli or Gram-negative bacilli ingested with food (3).
This study shows a small impairment of microbial CR in four out of six volunteers who used cephradine 1 g daily. So, even at this low dosage cephradine is not completely safe. It should be noted however, that absence of influence on CR has not yet been proven for any antibiotic administered in therapeutic dosage. The increase in the faecal concentration of resistant aerobic flora following cephradine certainly is much less than following amoxycillin (3). Therefore, cephradine is preferable to amoxycillin for the treatment of urinary tract infections caused by Gram-negative bacilli. However, we consider this low dosage not suitable for treatment of other than urinary tract infections.

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Table I. Influence of cephradine on aerobic faecal flora, expressed as log_{10} median faecal concentration (cfu/g) and range

Volunteer number	Gram-negative bacilli A	Gram-negative bacilli B	Gram-positive cocci A	Gram-positive cocci B	Yeasts A	Yeasts B
1	6 (4-7)	4(4-7)^{ns}	4(<2-5)	4(2-5)^{ns}	3(2-4)	3 (3-4)^{ns}
2	3(<2-5)	4(2-5)^{ns}	4 (3-5)	3(<2-4)$^{--}$	<2(<2-3)	3(<2-3)$^{+}$
3	7 (4-8)	8(6-9)^{ns}	3 (3-5)	4(3-6)^{ns}	<2(<2-4)	<2(<2-4)^{ns}
4	6 (5-7)	6(4-7)^{ns}	3(<2-5)	3(2-6)^{ns}	<2(<2-2)	<2(<2-3)^{ns}
5	6 (5-6)	6(6-7)$^{++}$	4 (3-5)	3(<2-3)$^{--}$	3(2-3)	3 (3-4)$^{+}$
6	5 (3-6)	6(5-7)^{ns}	5 (3-6)	5(<2-7)^{ns}	2(<2-4)	3 (3-4)$^{+}$

A : Pretreatment period (n=10). B : Treatment period (n=10).
$^{++}$ or $^{--}$: $P < 0.01$; $^{+}$ or $^{-}$: $P < 0.05$; ^{ns} : $P > 0.05$, two sided significance of a difference in concentration between periods A and B.

Table II. Influence of cephradine on faecal concentration[a] of Gram-negative bacilli other than Escherichia coli (secondary colonization)[b]

Volunteer number	Pre-treatment Period	Treatment Period
1	4 (<2-5)	3 (<2-4)^{ns}
2	<2 (<2-2)	<2(<2-<2)^{ns}
3	<2 (<2-3)	7 (4-9)$^{++}$
4	<2 (<2-3)	<2 (<2-4)^{ns}
5	<2 (<2-4)	5 (<2-7)$^{++}$
6	<2 (<2-4)	<2 (<2-4)^{ns}

[a] : Log_{10} median faecal concentration (cfu/g) and range.
[b] : Challenge strain not taken into account.
$^{++}$: $P < 0.01$; ^{ns} : $P > 0.05$, two sided significance of a difference in concentration between pre-treatment period and treatment period.

Table III. Influence of cephradine on colonization of the bowel by the challenge strain[a]

Volunteer number	Pre-treatment Period C_{max}[b]	Pre-treatment Period duration[c]	Treatment Period C_{max}[b]	Treatment Period duration[c]
1	10^4	2	10^3	3
2	10^3	2	$<10^1$	0
3	$<10^1$	0	$<10^1$	0
4	10^4	2	10^1	1
5	10^3	1	10^3	1
6	10^1	1	10^6	5

[a] : Oral administration of 10^9 cfu of Klebsiella pneumoniae, ($MIC_{cephradine}$ = 32 mg/l).
[b] : Maximal faecal concentration (cfu/g).
[c] : Number of days with positive faecal samples.

Table IV. Influence of cephradine on indicators of impairment of colonization resistance of the bowel

Volunteer number	Faecal concentration of : Gram-negative bacilli	Gram-positive cocci	Yeasts	SSC[a]	Challenge strain
1	-	-	-	-	-
2	-	-	+	-	-
3	-	-	-	+	-
4	-	-	-	-	-
5	+	-	+	+	-
6	-	-	+	-	+

[a] : Spontaneously acquired secondary colonization.
- : No increase.
+ : Increase.

CHAPTER IX

THE CONTRIBUTION OF ESCHERICHIA COLI TO MICROBIAL COLONIZATION RESISTANCE

E.J. Vollaard[a], H.A.L. Clasener[b] and A.J.H.M. Janssen[b]

Departments of [a]Pharmacy and [b]Microbiology, Canisius-Wilhelmina Hospital, St. Annastraat 289, 6525 GT Nijmegen, the Netherlands

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Abstract

The contribution of *Escherichia coli* to the microbial colonization resistance (CR) of the bowel was investigated in six healthy volunteers. *E. coli* was eliminated from faeces by the adminstration of a low dose (20 mg) of pefloxacin. This did not cause an increase in the faecal concentration of aerobic Gram-positive cocci or yeasts, nor did it facilitate colonization of the bowel by a pefloxacin-resistant challenge strain of *Klebsiella pneumoniae*. Therefore, *E. coli* does not appear to contribute to the microbial CR. After 18 days of pefloxacin, clindamycin 300 mg was administered twice daily for ten days. Clindamycin caused a significant increase in the faecal concentration of enterococci, yeasts and the *K. pneumoniae* challenge strain, indicating that the study design was suitable to demonstrate impairment of microbial CR if it occurred.

Introduction

The microbial CR limits both the number of aerobic species and their concentration in the digestive tract (1). Some authors presume that microbial CR is provided exclusively by the obligate anaerobic flora (2, 3). Others have shown that indigenous aerobic flora also contributes to CR in gnotobiotic animals (4, 5, 6). However, it should be noted that all animal experiments that give support to the contribution of aerobic flora in maintaining CR were performed in animals in whom the anaerobic flora that provides CR was absent or incomplete, causing an abnormally high faecal concentration of *E. coli*. So, although *E. coli* limits the concentration of other Gram-negative bacilli in the bowel of animals in whom the autochthonous anaerobic flora is not intact, the role of *E. coli* in normal animals and in man remains unknown. The present study was designed to investigate the contribution of *E. coli* to CR in human volunteers by studying the influence of its selective elimination on the faecal concentrations of indigenous aerobic flora of the bowel, and on the CR against a pefloxacin-resistant challenge strain of *Klebsiella pneumoniae*. However, because the anaerobic flora that provides CR is unknown, it is impossible to calculate the dosage of an antimicrobial agent that will achieve a faecal concentration above the

MIC of *E. coli* and, at the same time, below the MIC of the anaerobic flora that provides CR. Therefore, we decided to use the lowest effective dosage of an agent that is much more active against *E. coli* than against known species of anaerobic flora. In this regard quinolones are suitable candidates. In an unpublished pilot study we found that the diffusible faecal concentration of pefloxacin is the biologically active faecal concentration, and that pefloxacin 800 mg daily gives a diffusible faecal concentration of about 160 mg/l. Because the MIC of most strains of *E. coli* is below 4 mg/l, we decided to aim at a diffusible faecal concentration of 4 mg/l. Therefore a dosage of 20 mg pefloxacin was appropriate. To demonstrate that our study design was suitable to show a significant increase in aerobic flora in case of impairment of CR, the pefloxacin-period was followed by a period in which we administered clindamycin. If elimination of *E. coli* in the pefloxacin period would not facilitate colonization by the challenge strain, this could be ascribed to possible sub-MIC effects of pefloxacin. To exclude this possibility, continued administration of pefloxacin occurred during the first five days of the clindamycin-period.

Methods

Study design

During the study, daily faecal samples were analyzed for their concentration of Gram-negative bacilli, enterococci and yeasts. Daily production of faeces was an admission criterion for the volunteers. If more than one motion was passed, a sample of the first motion was used for analysis. The data from the first day of the two antibiotic periods were not used for analysis, because influence of antibiotics on these samples will be small or absent. The sample of the first morning after termination of clindamycin was used as the last treatment sample of that period.

The study was divided in four periods:

Period A, a pre-treatment period of ten days, served to investigate the normal concentration of aerobic flora and the CR against a pefloxacin-resistant challenge strain of *K. pneumoniae*.

In *period B*, pefloxacin 20 mg was administered for 18 days, in order to investigate the influence of elimination of indigenous Gram-

negative bacilli (especially *E. coli*) on the CR against enterococci, yeasts and pefloxacin-resistant species of Gram-negative bacilli. The duration of this treatment period was longer than the ten days we have advocated before (7), because several days pass before *E. coli* is eliminated from faeces and it was our intention to study a period of ten days in which the volunteers would be free of indigenous Gram-negative bacilli.

In *period C*, clindamycin was administered for ten days in order to investigate the influence of disturbance of the CR on the faecal concentration of indigenous aerobic flora and on the CR against the challenge strain. During the first five days of this period administration of pefloxacin 20 mg daily was continued, to exclude possible sub-MIC effects of pefloxacin as a cause of failure of the challenge strain to colonize the bowel during period B.

In *period D*, the post-treatment period, no antibiotics were administered. In this period, analysis of daily faecal samples was continued in each volunteer until the faecal concentration of the challenge strain was below detection limit for at least three days.

Challenge with 10^{11} cfu of a pefloxacin resistant strain of *K. pneumoniae* was performed on day 3 of the periods A and C, and on day 8 of period B. During periods B and C, the faecal concentration of pefloxacin was analyzed by an agar diffusion method.

Volunteers.

Six healthy volunteers, one female (age 24) and five male (age 23, 25, 39, 40 and 47) participated in the study. The volunteers had not used antibiotics for at least one month and gave written informed consent before the start of the study. Permission for this study was obtained from the ethics committee of the Canisius-Wilhelmina Hospital.

The female volunteer did not participate in part C of the study, because of the possibility that clindamycin may interfere with oral anticonception (8).

Drugs

Pefloxacin capsules 20 mg, prepared in the hospital pharmacy, were taken once daily with lunch. Two 150 mg capsules of clindamycin were taken twice daily after lunch and evening dinner.

Challenge.
As a challenge strain we used a strain of *K. pneumoniae*, highly resistant to pefloxacin (MIC=56 mg/l).
For administration, colonies of the challenge strain grown overnight on solid medium were suspended in a glass of water and drunk after a warm meal.

Bacteriology.
Serial 1/10 dilutions of faeces were made in Thioglycollate medium (BBL). One-microlitre volumes of each dilution were inoculated on to solid media to isolate Gram-negative bacilli (eosin methylene-blue lactose sucrose agar, Merck), the *K. pneumoniae* challenge strain (eosin methylene-blue lactose sucrose agar, Merck, with pefloxacin 5 mg/l), amoxycillin-resistant Gram-negative bacilli (5% sheep blood in blood agar base, Oxoid, with amoxycilin 10 mg/l), enterococci and streptococci (5% sheep blood in blood agar base, Oxoid, with nalidixic acid 50 mg/l), and yeasts (Sabouraud dextrose agar, Gibco, with chloramphenicol 125 mg/l). The solid media were also inoculated with 100-microlitre volumes of the first 1/10 dilutions of faeces, lowering the detection limit to 100 micro-organisms per gram of faeces. Concentrations were expressed as the logarithms to the base of 10 of the counts per gram of faeces, rounded up or down to whole numbers. Micro-organisms were identified by standard laboratory methods.

Faecal concentration of antibiotics
The diffusible faecal concentration of pefloxacin was determined by an agar-diffusion method, using a solid medium seeded with *E. coli* (ATCC 25922). The MIC of this strain for pefloxacin is 0.25 mg/l. The diameter of the inhibition zone was compared with the diameters of the inhibition zones around standard solutions of pefloxacin in normal saline.

Statistics
We analyzed the influence of the antibiotics on the faecal concentration of Gram-negative bacilli, enterococci and yeasts. Because the faecal concentration of antibiotics and the susceptibility to antibiotics of the faecal flora that provides CR may differ between volunteers, the data were analyzed for each volunteer individually,

using single case statistical techniques (7).
The absence of serial dependency of the data was verified by computing auto-correlations for time lags of one to four days for each series of data. For time lags of two to four days the correlation coefficients scattered around zero, as expected under the null-hypothesis of zero-correlation. For a time lag of one day a slight autocorrelation ($r = 0.24$) did not disprove sufficiently the prerequisite independence of observations. Thus the Mann-Whitney-Wilcoxon test could be employed to evaluate the influence of the antibiotics on the faecal concentrations of Gram-negative bacilli, enterococci and yeasts. The counts in the pre-treatment samples were used as a baseline.

Results

The median level and range of the faecal concentrations of Gram-negative bacilli, enterococci and yeasts are shown in Tables I, II and III.
The maximal faecal concentrations of the challenge strain and the number of days this strain was detectable after challenge are shown in Table IV.

Period A.
Except in volunteer 6, *E. coli* was the dominant species of Gram-negative bacilli in all faecal samples. Gram-negative bacilli other than *E. coli* were detected in eight, zero, two, zero, three and two samples of volunteers 1-6 respectively. All these species were susceptible to pefloxacin. In volunteer 6 the concentration of Gram-negative bacilli was below detection limit in eight of ten samples. In the other two samples 10^5 cfu *Enterobacter cloacae* and 10^2 cfu *K. pneumoniae* were found respectively. In volunteer 6, bèta-haemolytic streptococci group G outnumbered enterococci. In the other volunteers the concentration of enterococci was higher than the concentration of streptococci. The faecal concentration of the challenge strain dropped below detection limit within 4 days in all volunteers.

Period B.
From three days after the start of administration of pefloxacin the diffusible faecal concentration of all samples of all volunteers was between 1 and 6 mg/l. The median diffusible faecal concentration of pefloxacin in volunteers 1-6 respectively was 4, 4, 2, 4, 3 and 2 mg/l. *E. coli* was eliminated from faeces of volunteers 1-5 on days 4, 4, 3, 7 and 5 respectively. (In volunteer 6 Gram-negative bacilli were even not detectable before start of pefloxacin). Secondary Gram-negative bacilli other than the challenge strain were detectable only in the first three treatment samples in volunteers 1 and in one treatment sample (day 4) in volunteer 6. In volunteer 5 recolonization with *E. coli* occurred at day 8 and continued for the rest of period B. The MIC of this strain of *E. coli* for pefloxacin was 3 mg/l, equal to the diffusible faecal concentration in this volunteer, which fluctuated between 2 and 4 mg/l (median 3 mg/l).
The elimination of *E. coli* in period B did not facilitate colonization by the challenge strain compared to the pre-treatment period. As in the pre-treatment period, all samples were negative within four days. The faecal concentration of enterococci and yeasts did not increase significantly in any volunteer. In volunteer 3 the concentration of bèta-haemolytic streptococci group B became higher than the concentration of enterococci in period B, but did not increase above the concentration of enterococci in the pre-treatment period. In volunteer 6, the faecal concentration of streptococci remained higher than the concentration of enterococci, but at the same level as in the pre-treatment period.

Period C.
The faecal concentration of the challenge strain in the first sample after administration (10^7 cfu/gram in one volunteer and 10^8 cfu/gram in the other four) was much higher than in period A and B and stable, high level colonization persisted in all five volunteers. The faecal concentration of the challenge strain in period C was significantly higher than the faecal concentration of Gram-negative bacilli in periods A and B.
Although administration of clindamycin did not influence the faecal concentration of pefloxacin, it caused a significant increase in the faecal concentration of *E. coli* in volunteer 5, and recolonization with Enterobacteriaceae occurred in volunteers 4 and 6 before ad-

ministration of pefloxacin was terminated. *E. coli* (MIC 0,25 - 0,5 mg/l) was detectable again at day 4 in volunteer 4. Volunteer 6 was colonized at day two of this period with a spontaneously acquired strain of *K. pneumoniae* (MIC 1 mg/l), different from the challenge strain. In most samples of volunteers 2 and 4 the concentration of streptococci and enterococci could not be measured because of overgrowth by the challenge strain. In the other volunteers the faecal concentration of enterococci increased significantly. In this period enterococci outnumbered streptococci in all volunteers, including volunteers 3 and 6. The faecal concentration of yeasts increased significantly in volunteers 2-5, but not in volunteer 6.

Period D.
Recolonization with *E. coli* occurred at day 2 of period D in volunteers 2, 3 and 6. (The other volunteers were recolonized before). The challenge strain dropped below the detection limit at days 23, 27, 18, 11 and 9 respectively in volunteers 2-6. Volunteer 6, who was free from Gram-negative bacilli in most faecal samples in the pre-treatment period, remained colonized with both *E. coli* ($>10^7$ cfu/g) and the spontaneously acquired *K. pneumoniae* ($> 10^3$ cfu/g) up to the last sample we investigated (day 42 after termination of clindamycin).

Discussion

Colonization resistance
With normal food we probably ingest between a few thousand and a few million cfu of Gram-negative bacilli, especially *via* salads (9, 10). So, a challenge dose of 10^{11} cfu *K. pneumoniae* exceeds the normal intake of Gram-negative bacilli. Nevertheless, in the pre-treatment period this challenge dose was eliminated from the bowel within four days in all six volunteers. This demonstrates the high efficacy of CR of the bowel against exogenous micro-organisms. This powerful mechanism of defence appears to collapse completely following administration of clindamycin, but not at all following a low dosage of pefloxacin.
The simultaneous increase of the faecal concentration of Gram-negative bacilli, enterococci and yeasts in volunteers 3 and 5

following clindamycin (period C) demonstrates that none of these three groups of aerobic micro-organisms limits the concentration of the other. This supports the hypothesis that the limiting action of autochthonous flora on the faecal concentration of these three groups of aerobic micro-organisms is provided by anaerobic flora.
The faecal concentration of enterococci and of yeasts did not increase in any volunteer following elimination of *E. coli* in period B, demonstrating that indigenous Gram-negative bacilli do not contribute to the limiting action of the normal flora on the faecal concentration of other groups of aerobic micro-organisms. This is supported by the observation that the CR against enterococci, yeasts and the challenge strain was maintained in volunteer 6 during the pre-treatment period, although this volunteer was not colonized by Gram-negative bacilli during that period. The two samples positive for Gram-negative bacilli in this volunteer during the pre-treatment period were probably caused by micro-organisms ingested with food, which also failed to colonize the bowel. So, this study shows that the indigenous Gram-negative bacilli do not provide CR against enterococci, yeasts or exogenous Gram-negative bacilli. Therefore, it is possible to eliminate Gram-negative bacilli from the bowel without impairing CR (selective decontamination).
It was our working-hypothesis that disturbance of the anaerobic flora that provides CR will cause a simultaneous increase of Gram-negative bacilli, aerobic Gram-positive cocci and yeasts, if antibiotic-resistant micro-organisms are present (7). However, in volunteer 6 the concentration of yeasts did not increase following clindamycin, although CR against Gram-negative bacilli and enterococci was strongly impaired. This shows that even strong disturbance of the anaerobic flora that provides CR against Gram-negative bacilli and aerobic Gram-positive cocci is not necessarily accompanied by increase of the faecal concentration of yeasts.

Secondary colonization
Contrary to our expectation, selective elimination of *E. coli* did not facilitate low level colonization of the bowel by other Gram-negative bacilli, called "substitution colonization" (1). Admittedly, our results do not prove that the phenomenon of substitution colonization does not exist, because we only used one challenge strain. It cannot be excluded that another strain of Gram-negative bacilli would have

succeeded in colonizing the bowel. However, following administration of clindamycin, our challenge strain appeared to be perfectly able to colonize the bowel in all five volunteers who participated in this part of the study, even in a concentration higher than the original concentration of *E. coli*. Therefore we conclude that it is unlikely that substitution colonization by secondary Gram-negative bacilli will occur very easily. So, the increase of secondary colonization following roxithromycin, co-trimoxazole and doxycyline in our previous studies (11, 12) was probably caused by disturbance of anaerobic flora that provides CR and not by "substitution colonization" following a decrease of the faecal concentration of *E. coli*. Further research is required to investigate whether the phenomenon of" substitution colonization" exists at all.

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Table I. Influence of antibiotics on faecal concentration* of Gram-negative bacilli

Volunteer	Period A	Period B	Period B'	Period C
1	8 (6-8)	<2 (<2-5)$^{--}$	<2 (<2-<2)$^{--}$	#
2	6 (6-8)	<2 (<2-4)$^{--}$	<2 (<2-<2)$^{--}$	8 (<2-9)$^{++}$
3	6 (2-7)	<2 (<2-6)$^{--}$	<2 (<2-<2)$^{--}$	8 (<2-9)$^{++}$
4	7 (5-7)	<2 (<2-7)$^{--}$	<2 (<2-<2)$^{--}$	7 (<2-9)$^{++}$
5	7 (5-9)	4 (<2-6)$^{--}$	4 (2- 6)$^{--}$	8 (6-10)$^{++}$
6	<2(<2-5)	<2 (<2-3)^{ns}	<2 (<2-<2)^{ns}	8 (2-8)$^{++}$

* : Log_{10} median faecal concentration and range.
Period A : Pre-treatment period (n=10).
Period B : Pefloxacin 20 mg daily (n=17).
Period B': Last 10 samples of period B (E. coli absent in all samples of volunteers 1, 2, 3, 4 and 6).
Period C : Clindamycin 300 mg bid (n=10). (Pefloxacin 20 mg daily continued for the first five days).
: Volunteer did not participate in this period.
$^{++}$ or $^{--}$: $P < 0.01$; $^{+}$ or $^{-}$: $P < 0.05$; ^{ns} : $P > 0.05$, two sided significance of a difference in concentration between pre-treatment period and treatment period.

Table II. Influence of antibiotics on total faecal concentration of enterococci and streptococci

Volunteer	Period A	Period B	Period B'	Period C
1	4 (2-6)	4(<2-7)^{ns}	3(<2-)^{ns}	#
2	4 (3-7)	4(<2-6)^{ns}	4(<2-5)^{ns}	(a)
3	4 (2-5)	4(<2-5)^{ns}	4(<2-5)^{ns}	7(<2-8)$^{++}$
4	7 (5-8)	6(3-7)$^{-}$	6(4-6)^{ns}	(a)
5	4 (3-7)	5(3-8)^{ns}	5(3-8)^{ns}	8 (7-8)$^{++}$
6	5 (4-6)	5(<2-6)$^{-}$	5(3-6)^{ns}	8 (4-9)$^{++}$

(a) : Most samples overgrown by challenge strain.
Other legends as in Table I.

Table III. Influence of antibiotics on faecal concentration of yeasts

Volunteer	Period A	Period B	Period B'	Period C
1	<2 (<2-3)	<2 (<2-3)^{ns}	<2 (<2-2)^{ns}	#
2	2 (<2-3)	2 (<2-3)^{ns}	2 (<2-3)^{ns}	4 (3-6)$^{++}$
3	3 (<2-4)	3 (<2-5)^{ns}	3 (<2-4)^{ns}	5 (2-5)$^{+}$
4	2 (<2-3)	2 (<2-3)^{ns}	2 (<2-3)^{ns}	5 (4-5)$^{++}$
5	2 (<2-3)	3 (<2-5)^{ns}	3 (<2-5)^{ns}	3 (2-4)$^{++}$
6	<2 (<2-2)	<2 (<2-3)^{ns}	<2 (<2-3)^{ns}	<2(<2-2)^{ns}

Legends as in Table I.

Table IV. Influence of antibiotics on colonization by the challenge strain[a]

Volunteer number	Period A C_{max}[b]	Period A duration[c]	Period B C_{max}	Period B duration	Period C+D C_{max}	Period C+D duration
1	10^5	3	10^4	2	#	
2	10^4	3	10^3	1	10^9	30
3	10^6	2	10^3	2	10^9	34
4	10^6	2	10^6	2	10^9	25
5	10^6	2	10^6	3	10^{10}	18
6	$<10^2$	0	$<10^2$	0	10^9	16

[a] : 10^{11} cfu Klebsiella pneumoniae, resistant to pefloxacin (MIC = 56 mg/l).
Periods A, B and C as in Table I. ; Period D : Post-treatment period.
[b] : Maximal faecal concentration (cfu/g).
[c] : Number of days with positive faecal samples.
: Volunteer did not participate in this period.

CHAPTER X

DECONTAMINATION OF THE BOWEL BY INTRAVENOUS ADMINISTRATION OF PEFLOXACIN

E.J. Vollaard[a], H.A.L. Clasener[b] and A.J.H.M. Janssen[b].

Departments of [a]Pharmacy and [b]Microbiology, Canisius-Wilhelmina Hospital, St. Annastraat 289, 6525 GT Nijmegen, The Netherlands

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Abstract

Intravenous administration of pefloxacin 400 mg twice daily rapidly decontaminated the bowel from Gram-negative bacilli in ten healthy volunteers. The faecal concentrations of enterococci and yeasts did not change significantly. Further, pefloxacin did not facilitate colonization of the bowel by a highly resistant challenge strain (*Klebsiella pneumoniae*, MIC = 56 mg/l).
The diffusible faecal concentration of pefloxacin was between 110 and 260 mg/l in all samples from day 3 of treatment onwards.
It is concluded that parenteral administration of pefloxacin is very effective for decontamination of the bowel from Gram-negative bacilli and provides reliable prophylaxis against colonization of the bowel by highly resistant Gram-negative bacilli ingested with food.

Introduction

Decontamination of the digestive tract from Gram-negative bacilli is useful in the prevention of infections in leukopenic patients (1), in patients on mechanical ventilation (2) and in patients with transurethral catheters (3). Until now, decontamination has usually been performed by oral administration of antimicrobial agents. Some patients who are treated with cytostatic agents, however, cannot tolerate an oral regimen because of severe vomiting. Further, in many patients who are receiving mechanical ventilation, decontamination of the bowel will be delayed if an oral regimen is used, because of initial paralytic ileus. Availability of a reliable parenteral regimen for decontamination of the bowel is therefore highly desirable.
Decontamination of the bowel from Gram-negative bacilli has been achieved with parenteral administration of aztreonam or temocillin (4, 5). Both drugs, however, are completely inactivated in faeces of some volunteers (5). Enzymatic inactivation in faeces has also been described for ceftriaxone (6). Pefloxacin has two possible advantages for parenteral decontamination of the bowel. Firstly, it attains high concentrations in faeces following oral administration, although it is nearly completely absorbed (7). Equally high faecal concentrations can therefore be expected following intravenous administration.

Secondly, enzymatic inactivation of quinolones in faeces has never been reported.
We therefore investigated the influence of parenteral administration of pefloxacin on the normal aerobic faecal flora, and on colonization of the bowel by a highly resistant challenge strain.

Methods

Study design
In five volunteers (1-5), daily faecal samples were analyzed for their concentration of Gram-negative bacilli, enterococci and yeasts during a treatment period of five days and on one day before (day 0) and one day after treatment (day 6).
Five other volunteers (6-10) had faecal samples analyzed in the same way, but were also given 10^{11} cfu of a pefloxacin-resistant strain of *Klebsiella pneumoniae* in the pre-treatment period and in the treatment period, and the faecal concentration of pefloxacin of these volunteers was analyzed.

Volunteers
Five healthy volunteers (two female, three male), aged 27 - 53 years (median age 42 years) participated in the first part of the study, and five other volunteers (all male), aged 31 - 47 years (median age 45 years) in the second part of the study. The volunteers had not received antibiotics for at least one month before the start of the study. Written informed consent was obtained from the volunteers and permission for the study was granted by the ethics committee of the Canisius-Wilhelmina Hospital.

Drugs
Pefloxacin was administered intravenously twice daily at 09.00 and 17.00 hours. One ampoule of pefloxacin 400 mg was added to dextrose 5% infusion fluid and infused through a Venflon needle.

Challenge
As a challenge strain for the second set of volunteers (6-10), we used the strain with the highest resistance of a species of Enterobacteriaceae that we could obtain. The MIC for pefloxacin of this

strain (of *K. pneumoniae*) was 56 mg/l, but it was highly sensitive to cefotaxime and co-trimoxazole. The challenge strain could therefore easily be isolated from faeces with selective culture medium. At the same time, effective antibiotics were available in case of infection by the challenge strain. The challenge strain was administered in a dosage of 10^{11} cfu at day 12 before the start of pefloxacin and at the second day of pefloxacin administration. Colonies of the challenge strain, grown overnight on solid medium, were suspended in a glass of water and drunk after a warm meal to bypass the acidic environment of the stomach.

Bacteriology

One gram of faeces was suspended in 9 ml of Thioglycollate medium (BBL), and serial 1/10 dilutions were made. One microlitre-volumes of each dilution were inoculated on to solid media to isolate Gram-negative bacilli (eosin methylene-blue lactose sucrose agar, Merck), the *K. pneumoniae* challenge strain (eosin methylene-blue lactose sucrose agar, Merck, with pefloxacin 5 mg/l), enterococci (5% sheep blood in blood agar base, Oxoid, with nalidixic acid 50 mg/l), and yeasts (Sabouraud dextrose agar, Gibco, with chlor-amphenicol 125 mg/l). The solid media were also inoculated with 100-microlitre volumes of the first 1/10 dilution of faeces, lowering the detection limit to 100 micro-organisms per gram of faeces. Concentrations were expressed as the logarithms to the base 10 of the counts per gram of faeces, rounded up or down to whole numbers. Micro-organisms isolated were identified by standard laboratory methods.

The faecal concentration of pefloxacin was determined by an agar-diffusion method. Faeces was put in a well in a solid medium seeded with *Escherichia coli* (ATCC 25922). The MIC of this strain for pefloxacin is 0.25 mg/l. The diameter of the inhibition zone was compared to the diameters of the inhibition zones around standard solutions of pefloxacin in saline in wells in the same agar plate. The concentration found was called the diffusible faecal concentration.

Statistics

Friedman's test was employed for evaluation of the influence of pefloxacin on the counts of micro-organisms in faeces. The counts in the samples of the day before the first administration of pefloxacin

(day 0) were used as a base-line. The data of day 1 were not analyzed, because these samples may be influenced by drug treatment if they are produced after the first dosage of pefloxacin. The morning-samples after the last day of pefloxacin-treatment were counted as the last treatment samples.

Results

Pefloxacin rapidly eliminated indigenous Gram-negative bacilli from faeces. The median time for elimination of Gram-negative bacilli was two days (Table I). Gram-negative bacilli were no longer detectable after one day in volunteers 4, 6 and 10, after two days in volunteers 3, 5, 8 and 9, after three days in volunteer 7, and after five days in volunteer 2. In volunteer 1 the decontamination proceeded irregularly. Gram-negative bacilli were not detectable at days 3 and 5, but the concentrations of *E. coli* on days 4 and 6 were 10^4 and 10^3 cfu/g faeces respectively.

Administration of pefloxacin did not facilitate colonization of the bowel by the challenge strain (*K. pneumoniae*, Table II).

Pefloxacin did not cause a significant change in the faecal concentration of enterococci ($P = 0.11$) or yeasts ($P = 0.70$), (Tables III and IV).

The diffusible faecal concentration of pefloxacin increased up to a plateau-level at about day 4. The median faecal concentration between days 4 and 6 was 140, 238, 150, 175, and 160 mg/l in volunteers 1-5 respectively. From day 3 onwards, the faecal concentration was between 110 and 260 mg/l in all samples (Table V).

Discussion

Intravenous administration of pefloxacin, in a standard dosage of 400 mg twice daily, proved to be suitable for decontamination of the bowel from indigenous Gram-negative bacilli and could be considered for decontamination in high risk patients unable to tolerate an oral regimen.

Adminstration of pefloxacin did not facilitate colonization of the bowel following a challenge dose with a highly resistant strain of

K. pneumoniae (MIC = 56 mg/l). In a previous study, the same strain of *Klebsiella* caused high-level colonization of the bowel in volunteers in whom colonization resistance (CR) was impaired by administration of clindamycin (8). Therefore, this strain seemed suitable for demonstration of impairment of CR. However, in this study the failure of the challenge strain to colonize the bowel does not prove that pefloxacin left CR unimpaired, because the diffusible faecal concentration of pefloxacin was higher than the MIC of the challenge strain in all volunteers. If we assume that the diffusible faecal concentration represents the active concentration in the bowel, then we were unable to investigate the influence of pefloxacin 800 mg daily on CR against Gram-negative bacilli, as a Gram-negative bacillus with a MIC higher than the diffusible faecal concentration of pefloxacin was not available.

Pefloxacin did provide effective prophylaxis against colonization of the bowel by the most resistant strain available, to emphasize the excellent safety-margin of pefloxacin for prophylaxis of endogenous Gram-negative infections.

The data for the diffusible faecal concentrations of pefloxacin are within a very small range, after the plateau-level has been attained at day 4. From the first 24 h of treatment, the diffusible faecal concentration of pefloxacin was far above the MIC of normally occurring Gram-negative bacilli. Parenteral administration of pefloxacin thus provides highly reliable concentrations of antibiotic in the bowel.

Increase of the faecal concentration of yeasts was not observed for this group of volunteers. A larger number of pre-treatment and treatment samples is required however, to investigate whether CR against yeasts remains unaffected in all of the volunteers individually (9).

We conclude that parenteral administration of pefloxacin is a very suitable method for the elimination of Gram-negative bacilli from the bowel, and that it provides reliable prophylaxis against colonization of the bowel by Gram-negative bacilli ingested with food. In the case of gastric retention in mechanically ventilated patients, however, gastric decontamination may be a critical factor in the prevention of infections. It is not known whether gastric decontamination in those patients will be achieved by parenteral administration of pefloxacin. So, as yet, it is advisable to decontaminate the gastric contents in those patients by local administration of antimicrobial agents as well.

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Table I. Influence of pefloxacin[a] on the faecal concentration[b] of Gram-negative bacilli[c]

Volunteer number	Day : 0	2	3	4	5	6
1	7	3	<2	4	<2	3
2	9	4	4	2	2	<2
3	5	2	<2	<2	<2	<2
4	6	<2	<2	<2	<2	<2
5	6	3	<2	<2	<2	<2
6	5	<2	<2	<2	<2	<2
7	7	6	3	<2	<2	<2
8	4	2	<2	<2	<2	<2
9	7	7	<2	<2	<2	<2
10	2	<2	<2	<2	<2	<2

[a] : 400 mg intravenously twice daily day 1-5.
[b] : Log_{10} concentration (cfu/g).
[c] : Excluding the challenge strain (in volunteers 6-10).

Table II. Influence of pefloxacin on colonization of the bowel by the challenge strain[a]

Volunteer number	Pretreatment period C_{max}[b]	duration[c]	Treatment period C_{max}[b]	duration[c]
6	10^3	1	$<10^2$	0
7	$<10^2$	0	10^3	1
8	10^4	2	$<10^2$	0
9	10^4	2	10^5	3
10	10^4	1	$<10^2$	0

[a] : Oral administration of 10^{11} cfu K.pneumoniae 12 days before treatment with pefloxacin and on the second day of the pefloxacin period.
[b] : Maximal faecal concentration (cfu/g).
[c] : Number of days with positive faecal samples.

Table III. Influence of pefloxacin[a] on the faecal concentration[b] of enterococci

Volunteer number	Day : 0	2	3	4	5	6
1	5	3	5	<2	5	<2
2	6	6	5	2	2	<2
3	5	6	6	5	5	5
4	4	4	4	4	4	2
5	5	5	4	5	5	4
6	3	<2	<2	<2	<2	2
7	3	6	3	<2	<2	3
8	5	<2	<2	<2	3	3
9	5	4	5	5	3	3
10	4	4	3	4	3	4

[a] : 400 mg intravenously twice daily day 1-5.
[b] : Log_{10} concentration (cfu/g).

Table IV. Influence of pefloxacin[a] on the faecal concentration[b] of yeasts

Volunteer number	Day : 0	2	3	4	5	6
1	<2	<2	<2	<2	3	<2
2	2	<2	<2	<2	2	2
3	<2	3	3	3	3	4
4	<2	<2	<2	<2	<2	<2
5	<2	4	<2	3	2	3
6	3	3	2	2	3	2
7	3	3	3	3	3	3
8	3	3	3	4	3	4
9	<2	<2	<2	<2	<2	<2
10	2	2	2	<2	2	2

[a] : 400 mg intravenously twice daily day 1-5.
[b] : Log_{10} concentration (cfu/g).

Table V. Diffusible faecal concentration of pefloxacin[a]

volunteer no.	day 1	day 2	day 3	day 4	day 5	day 6
6	0	92	125	140	130	150
7	0	34	110	125	260	238
8	0	100	122	170	150	155
9	0	110	118	180	154	175
10	0	90	150	160	150	218

[a] : mg pefloxacin gram faeces, measured by an agar-diffusion method.

CHAPTER XI

INFLUENCE OF PEFLOXACIN ON MICROBIAL COLONIZATION RESISTANCE IN HEALTHY VOLUNTEERS

E.J. Vollaard[a], H.A.L. Clasener[b] and A.J.H.M. Janssen[b].

Departments of [a]Pharmacy and [b]Microbiology, Canisius-Wilhelmina Hospital, St. Annastraat 289, 6525 GT Nijmegen, The Netherlands

Abstract

The influence of pefloxacin, 400 mg twice daily for ten days, on microbial colonization resistance (CR) was investigated in six healthy volunteers. Increase in the faecal concentration of Gram-negative bacilli, aerobic Gram-positive cocci and yeasts, and facilitation of colonization by a highly resistant challenge strain (*Klebsiella pneumonia*, MIC = 56 mg/l), were used as indicators for disturbance of the flora that provides CR. Because the faecal concentration of pefloxacin during treatment is higher than the MIC of the challenge strain, challenge was repeated in the post-treatment period.

Because the influence of antibiotics on CR may differ between volunteers, data were analyzed for the volunteers individually.

The median faecal concentrations in the samples between day 5 and the first post-treatment day, were between 119 and 231 mg/l (median value 154 mg/l).

Gram-negative bacilli were rapidly eliminated from faeces in all volunteers, and the faecal concentration of aerobic Gram-positive cocci decreased in five of six volunteers.

In three of six volunteers, disturbance of CR-flora was indicated by a significant increase in the faecal concentration of yeasts. In two of them, this was confirmed by facilitation of colonization by the challenge strain in the post-treatment period. In the third one this did not occur, because CR was restored too rapidly.

It is concluded that pefloxacin is a highly reliable agent for elimination of Gram-negative bacilli from faeces. In a dosage of 400 mg twice daily pefloxacin impairs CR in some volunteers, but bacterial overgrowth is prevented by the high faecal concentration of pefloxacin.

Introduction

Quinolones have been used successfully for elimination of Gram-negative bacilli from the bowel in neutropenic patients (1), in patients who are mechanically ventilated (2, 3), and in patients with transurethral catheters (4). It is usually assumed that decolonization by quinolones is "selective" (5), *id est* that quinolones do not disturb

the anaerobic flora that provides CR (6). However, an increase in the faecal concentration of *Candida* spp. has been found following ciprofloxacin (7) and ofloxacin (8), casting doubt on sparing of the CR-flora by these quinolones.

It has been postulated that "selective" elimination of Gram-negative bacilli from the bowel is not possible, because elimination of aerobic flora would increase the oxygen tension in the bowel, causing elimination of the most sensitive anaerobic species (9). This might explain the discrepancy between the observed increase of the faecal concentration of yeasts following antimicrobial agents that are supposed to spare the anaerobic flora that provides CR.

However, using a sensitive method of establishing impairment of CR, we did not find a decrease in CR against a resistant challenge strain of *Klebsiella pneumoniae*, enterococci or yeasts in volunteers following elimination of *Escherichia coli* with a low dosage (20 mg daily) of pefloxacin (10). Hence, increase of the faecal concentration of yeasts demonstrates disturbance of the anaerobic flora that provides CR.

Bacterial overgrowth of the bowel following quinolones has not been reported, but this might be caused by the absence of strains that are sufficiently resistant to the very high faecal concentration of quinolones, in stead of by sparing of the anaerobic flora that provides CR. In a pilot study, we found that a highly resistant challenge strain of *K. pneumoniae* (MIC for pefloxacin 56 mg/l), did not colonize the bowel in a volunteer who was treated with pefloxacin 400 mg twice daily. During treatment the diffusible faecal concentration of pefloxacin was about about 160 mg/l. However, when pefloxacin was stopped and the diffusible faecal concentration of pefloxacin dropped below the MIC, the challenge strain re-appeared spontaneously in faeces and remained detectable for about two weeks (Figure).

This challenge strain disappears from faeces within 5 days following a challenge dose, if CR is not disturbed (10, 11). So, although this strain is not sufficiently resistant to be used as a challenge for the influence of pefloxacin on CR against Gram-negative bacilli during treatment, this strain might be suitable shortly after treatment with pefloxacin. We decided to try this method in a larger number of volunteers. The study was designed to enable demonstration of a tenfold change in the faecal concentration of aerobic indigenous flora

with statistical significance (11). We also studied the diffusible faecal concentration of pefloxacin during and after treatment, and the time of re-colonization with Gram-negative bacilli after treatment.

Methods

Study design

Daily faecal samples were analyzed for their concentrations of Gram-negative bacilli, aerobic Gram-positive cocci and yeasts during a pre-treatment period, a treatment period and a post-treatment period of ten days each. An oral dose of 10^{11} cfu of a pefloxacin-resistant challenge strain of *K. pneumoniae* was administered on day 4 of the pre-treatment period, on day 2 of the treatment period, and on day 3 of the post-treatment period. The first challenge was intended to investigate the normal CR against Gram-negative bacilli. The second challenge was intended to repeat our previous observation that colonization by this strain does not occur if the diffusible faecal concentration is higher than the MIC of the challenge strain (Figure). The third challenge was intended to investigate whether the influence of pefloxacin on the autochthonous flora would facilitate colonization by a challenge strain when the diffusible faecal concentration dropped below the MIC of the challenge strain.

After the first ten days of the post-treatment period, faecal sampling was continued three times a week until two succeeding samples contained Gram-negative bacilli again and until the challenge strain was no longer detectable.

Volunteers

Six healthy volunteers, two female (both 44 years old) and four male (age 18, 28, 41 and 50 years) participated in the study. The volunteers had not received antibiotics for at least one month before the start of the study. Written informed consent was obtained from the volunteers and permission for the study was granted by the ethics committee of the Canisius-Wilhelmina Hospital.

Drug
Pefloxacin tablets 400 mg were administered twice daily after meals.

Challenge
As a challenge, we used the strain with the highest resistance of a species of Enterobacteriaceae that we could obtain. The MIC of this strain of *K. pneumoniae* for pefloxacin is 56 mg/l. Therefore, the challenge strain can easily be isolated from faeces with a selective culture medium. Since this strain is highly sensitive to cefotaxime and to co-trimoxazole, effective antibiotics are available in case of infection with the challenge strain. It has been shown before, that this strain is suitable for demonstration of impairment of CR (10).
For administration, colonies of the challenge strain grown overnight on solid medium were suspended in saline. The suspension was poured in a glass of water, and drunk after a warm meal, immediately followed by another glass of water. In this way, the bacterial suspension was expected to bypass the acidic environment of the stomach.

Bacteriology
Serial 1/10 dilutions of faeces were made in Thioglycollate medium (BBL). One microlitre-volumes of each dilution were inoculated on to solid media to isolate Gram-negative bacilli (Mc Conkey agar, Oxoid), amoxycillin-resistant Gram-negative bacilli (5% sheep-blood in blood agar base, Oxoid, plus amoxycillin 10 mg/l), quinolone-resistant Gram-negative bacilli (McConkey agar, plus norfloxacin 8 mg/l), enterococci and streptococci (5% sheep blood in blood agar base, plus nalidixic acid 50 mg/l), staphylococci (mannitol salt agar, bióMerieux), and yeasts (Sabouraud dextrose agar, Gibco, plus chloramphenicol 125 mg/l). The solid media were also inoculated with 100-microlitre volumes of the first 1/10 dilutions of faeces, lowering the detection limit to 100 micro-organisms per gram of faeces. The detection limit of the challenge strain was lowered to 10 micro-organisms per gram of faeces by inoculating 1 ml-volumes of the first 1/10 dilution of faeces on to extra-large Petri-dishes (diameter 14 cm) containing the selective medium.
Concentrations of micro-organisms were expressed as the logarithms to the base of 10 of the counts per gram of faeces, rounded up or

down to whole numbers.
Micro-organisms isolated were identified and their sensitivities to antibiotics was determined by standard laboratory methods.

Faecal concentration of pefloxacin
Faecal concentrations of pefloxacin were determined by an agar-diffusion method. Faeces was put in a well in a solid medium (isosensitest-agar, Oxoid), seeded with *E. coli* (ATCC 25922). The MIC of this strain for pefloxacin is 0.25 mg/l. The diameter of the inhibition zone was compared to the diameters of the inhibition zones around standard solutions of pefloxacin in normal saline in wells in the same bio-assay plate. The detection limit was about 1 mg/l. All samples were analyzed in triplicate and the median value was used. The concentration found in this way will be called the diffusible faecal concentration.

Statistics
The influence of pefloxacin on the faecal concentrations of aerobic micro-organisms in faeces was evaluated with the Mann-Whitney-Wilcoxon test for each volunteer individually, using single case statistical techniques as described before (10, 11).

Results

The diffusible faecal concentration of pefloxacin was higher than the MIC of the challenge strain on day 2 in five of six volunteers and on day 3 in all volunteers. The diffusible faecal concentration increased up to a plateau-level at about day 5. Between day 5 and the first post-treatment day, the median faecal concentration was 138, 134, 195, 119, 170, and 231 mg/l in volunteers 1-6 respectively. After the pefloxacin period, the faecal concentration of pefloxacin dropped rapidly, as in the Figure. The faecal concentration of pefloxacin dropped below detection limit (1 mg/l) in 7 days in three volunteers and in 4, 5 and 6 days in the other ones.
In the pre-treatment period, *E. coli* was the dominant species of Gram-negative bacilli in all volunteers. Colonization with Gram-negative bacilli other than *E. coli* (secondary colonization) was detected in 1, 4, 7, 6, 7, and 3 samples in volunteers 1-6

respectively, in a median concentration of 10^4 cfu/g (range 10^2 - 10^6 cfu/g).

The influence of pefloxacin on the faecal concentration of indigenous aerobic flora is shown in Table I.

Pefloxacin rapidly eliminated indigenous Gram-negative bacilli from faeces. The concentration was below detection limit (10^2 cfu/g) on day 2 in volunteers 2 and 6, and on day 3 in the other four.

Enterococci were the dominant species of aerobic Gram-positive cocci in all volunteers during the pre-treatment and the treatment period. The faecal concentration of aerobic Gram-positive cocci decreased significantly in volunteers 2-6, but not in volunteer 1. Pefloxacin did not influence the MIC of the enterococci that were isolated from faeces. During the pre-treatment period as well as the treatment and post-treatment period the MIC of nearly all enterococci was between 4 and 16 mg/l.

Pefloxacin caused a significant increase in the faecal concentration of yeasts in volunteers 3, 4, and 5.

In the post-treatment period, colonization of the bowel by the challenge strain was facilitated in volunteers 3 and 4, but not in volunteers 1, 2, 5 and 6 (Table II). In volunteer 3, the challenge strain re-appeared spontaneously in the first faecal sample of the post-treatment period and remained detectable in all samples up to day 6 and in a sample on day 10. In volunteer 4, faecal samples were positive for the challenge strain for eight days.

Gram-negative bacilli re-appeared in faeces at day 13, 5, 9, 13, 6 and 17 of the post-treatment period in volunteers 1-6 respectively. In four of six volunteers *E. coli* was the first species of Gram-negative bacilli to re-appear. In the other two volunteers *Klebsiella* (other than the challenge strain), *Acinetobacter* and *Citrobacter* spp. appeared first.

The influence of antimicrobial agents on CR can not be assessed by measuring the faecal concentration of anaerobic flora. The composition of the anaerobic flora that provides CR is unknown, therefore analysis of the concentration of the minor fraction of the anaerobic flora that is cultivatable yields inconclusive results. Also, antimicrobial agents might impair CR by diminishing the production of inhibitory substances, without reducing the concentration of the CR-flora.

Hence, disturbance of this flora has to be deduced from an increase in the intestinal concentration of 1) Gram-negative bacilli, 2) aerobic Gram-positive cocci or 3) yeasts, or by increase in secondary colonization (10, 11). It should be noted, that overgrowth by these micro-organisms is possible only if species are present that are resistant to the active concentration of the antibiotic in the bowel. Therefore, if the active faecal concentration of an antibacterial agent is high compared to the MIC of prevailing aerobic bacteria, the faecal concentration of yeasts remains as the best indicator of impairment of CR (10).

Between the fifth and the last day of treatment, the median faecal concentration of the volunteers was between 119 and 231 mg/l (median value 154 mg/l). In a previous study (12), the median faecal concentration following intravenous adminstration of pefloxacin in five volunteers was between 140 and 238 mg/l (median value 160 mg/l). The equivalence of the faecal concentration of pefloxacin following oral and parenteral administration confirms the excellent bio-availability of pefloxacin after oral administration and suggests that the influence of pefloxacin on the faecal flora will be similar following oral and parenteral administration.

The faecal concentration of yeasts was significantly increased in volunteers 3, 4 and 5, indicating disturbance of the anaerobic flora that provides CR. In volunteers 3 and 4 this was confirmed by facilitation of colonization with the *Klebsiella* challenge strain in the post-treatment period. In volunteer 3 the challenge strain re-appeared spontaneously in faeces in the first sample of the post-treatment period, following 6 negative faecal samples (in the treatment period). This demonstrates that the challenge strain had survived somewhere in the digestive tract, after the faecal samples

had become negative. A similar phenomenon occurred in the volunteer of the pilot trial (Figure). This shows that elimination of micro-organisms from faeces does not prove that they have been eliminated from the bowel. Probably the active concentration of pefloxacin is higher in the colon than in the rest of the bowel, enabling survival (for example in the ileum or in the caecum) of strains with a MIC below the diffusible faecal concentration of pefloxacin.

In volunteer 5, impairment of CR (as indicated by increase of the concentration of yeasts), could not be confirmed with the challenge strain in the post-treatment period. This was due to rapid reversal of impairment of CR. The faecal concentration of yeasts in this volunteer was between 10^4 and 10^6 cfu/g in the last four samples during pefloxacin and in the next two samples. From the third day of the post-treatment period onwards, all samples were below 10^4 cfu/g again.

In volunteer 3 also, the rapid reversal of CR would not have allowed detection of disturbance of CR if the challenge strain would not have re-appeared spontaneously in the first faecal sample after treatment. So, in general, impairment of CR following pefloxacin is reversed too rapidly to allow reliable detection of this impairment by challenge in the post-treatment period.

The faecal concentration of enterococci decreased significantly in five of six volunteers. In volunteers 2, 3, 4, and 6 the last two treatment samples were free of enterococci. However, elimination of enterococci from faeces appears to proceed much slower than elimination of Gram-negative bacilli. This slow elimination, together with the small number of samples and the short period of investigation used in most studies, might explain why influence of quinolones on faecal concentration of enterococci is not detected in most studies (5, 12).

We had expected that the MIC of the enterococci in the last treatment samples of the pefloxacin period would have been higher than the diffusible faecal concentration of pefloxacin. However, before, during and after treatment with pefloxacin, the MIC of the majority of isolates was between 4 and 16 mg/l. This is about 20 times lower than the diffusible faecal concentration of pefloxacin (119-231 mg/l in this study). On the other hand, faecal samples were rapidly cleared of the *K. pneumoniae* challenge strain in volunteers 3, 4 and

5 (although CR was impaired in these volunteers), though the diffusible faecal concentration of pefloxacin was only about three times higher than the MIC of the challenge strain. This confirms earlier observations that the diffusible faecal concentration is about equal to the concentration required for elimination (or at least reduction of the faecal concentration) of Gram-negative bacilli (10, Figure). Apparently, elimination of enterococci from faeces requires a higher faecal concentration of pefloxacin relative to their MIC, than elimination of Gram-negative bacilli. We have no explanation for this remarkable phenomenon.

We did not observe an increase in the faecal concentration of staphylococci, as was reported in a study with pefloxacin 400 mg three times daily (13). Possibly, that dosage provoked colonization with staphylococci by stronger impairment of CR than occurs following 400 mg twice daily.

The data of volunteers 1, 2 and 6 in this study show that the normal dosage of pefloxacin does not impair CR in all volunteers, and confirm our previous finding that it is possible to eliminate indigenous Gram-negative bacilli from faeces, without impairing CR against yeasts (10).

We conclude that pefloxacin is a highly reliable agent for elimination of Gram-negative bacilli from faeces. Although pefloxacin 400 mg twice daily impairs CR in part of the volunteers, this does not result in overgrowth with bacteria, because the active faecal concentration is too high during treatment, and because CR is restored rapidly after treatment.

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Table I. Influence of pefloxacin on the faecal concentration[a] of aerobic flora

Volunteer number	Gram-negative bacilli A	Gram-negative bacilli B	Gram-positive cocci A	Gram-positive cocci B	Yeasts A	Yeasts B
1	8(6-9)	<2 (<2-3)$^{--}$	4(3-6)	4(<2-5)^{ns}	<2(<2-2)	<2(<2- 5)^{ns}
2	6(5-7)	<2(<2-<2)$^{--}$	4(3-7)	3(<2-5)$^{-}$	3(<2-4)	3(2- 5)^{ns}
3	6(4-8)	<2 (<2-3)$^{--}$	5(3-5)	3(2-3)$^{--}$	<2(<2-2)	3(2- 3)$^{++}$
4	7(5-8)	<2 (<2-4)$^{--}$	6(3-8)	3(<2-5)$^{--}$	3(<2-4)	5(3- 6)$^{++}$
5	4(4-7)	<2(<2-<2)$^{--}$	6(6-7)	5(4-7)$^{--}$	<2(<2-3)	3(<2- 6)$^{++}$
6	6(3-8)	<2 (<2-4)$^{--}$	5(2-7)	3(<2-5)$^{-}$	<2(<2-2)	<2(<2-<2)^{ns}

[a] : Log_{10} median concentration (cfu/g) and range.
A : Pre-treatment period (n=10); B treatment period (n=10, except in volunteer 5; n = 9).
$^{++}$ or $^{--}$: P < 0.01 ; $^{+}$ or $^{-}$: P < 0.05 ; ^{ns} : P > 0.05 , two sided significance of a difference between periods A and B.

Table II. Influence of pefloxacin on colonization of the bowel by the challenge strain[a]

Volunteer number	Pre-treatment Period C_{max}[b]	Pre-treatment Period duration[c]	Treatment Period C_{max}[b]	Treatment Period duration[c]	Post-treatment Period C_{max}[b]	Post-treatment Period duration[c]
1	10^4	1	10^2	1	10^4	2
2	10^6	3	10^5	4	10^7	4
3	10^7	1	10^5	2	10^5	7
4	10^5	3	10^4	4	10^6	8
5	10^8	4	10^6	2	10^6	3
6	10^3	2	10^2	3	10^6	4

[a] : Oral administration of 10^{11} cfu of Klebsiella pneumoniae, MIC for pefloxacin 56 mg/l.
[b] : Maximal faecal concentration (cfu/g).
[c] : Number of days with positive faecal samples.

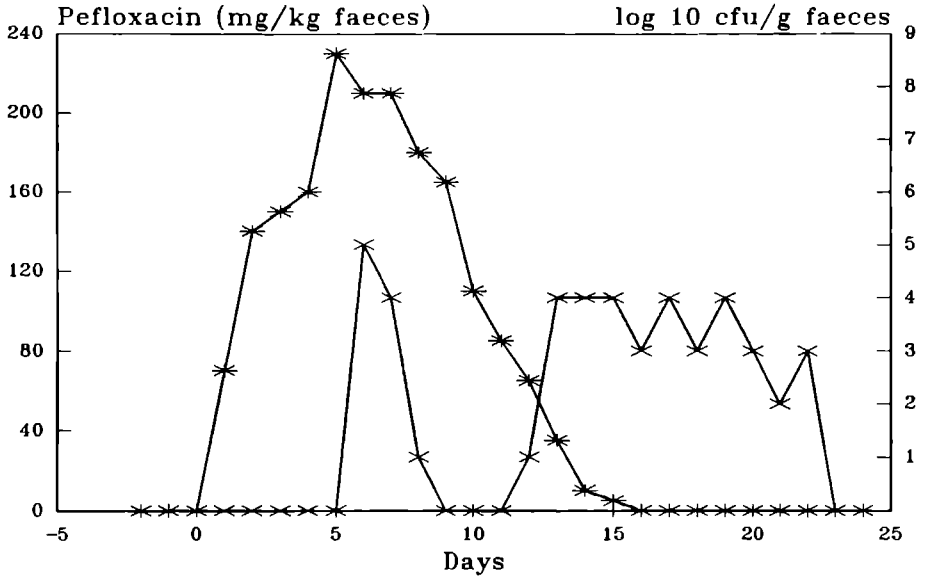

Figure. Influence of pefloxacin on the faecal concentration of Escherichia coli and of the Klebsiella pneumoniae-challenge strain in the pilot trial.
--* : Diffusible faecal concentration of pefloxacin.
x-x-x : Faecal concentration of the challenge strain, detection limit 10 cfu/g faeces.
Day 0 - Day 10 : 400 mg pefloxacin twice daily.
Day 5 : Oral adminstration of 10^{11} cfu Klebsiella pneumoniae.

CHAPTER XII

THE CONCEPT OF COLONIZATION RESISTANCE. A RE-APPRAISAL

Introduction

In 1987, we reviewed the data concerning the concept of microbial colonization resistance (CR). At that time, application of this concept had resulted in several papers reporting successful prevention of infections in leukopenic patients by selective decontamination, and selective decontamination in patients on mechanical ventilation had just been started. However, the concept was based on data obtained in animal experiments and had not been validated in man.

In the mean time a large number of new studies reporting successful application of selective decontamination have been published, and studies in man have been performed. These studies throw a new light on the concept and improve the understanding of the determinants of the influence of antimicrobial agents on the aerobic flora of the bowel.

So, it is time for a re-appraisal of the concept.

Autochthonous, normal and indigenous flora

Administration of antibiotics may disrupt the microbial ecology of the bowel, causing overgrowth by resistant micro-organisms and increasing the risk of superinfections (1). Apparently, microbial flora of the bowel protects against overgrowth by potentially pathogenic micro-organisms. The crucial question is : which part of the flora provides that protection ?

To tackle this question, Dubos made a distinction between the autochthonous flora, the normal flora and the indigenous flora of the bowel (2). In his words, ". *the autochthonous flora is a collection of micro-organisms that has achieved a symbiotic state with their host during a long period of evolutionary association*". This flora plays an essential role in the development and physiological activities of normal animals and man and provides the protection against colonization by potentially pathogenic flora. The autochthonous flora may differ between species, but is identical in all individuals of a species.

The "normal flora" is the sum of the autochthonous flora and of other micro-organisms that are so widely distributed and so adapted to the circumstances in the bowel that they succeed in colonizing

practically all members of a community.
"Indigenous flora" designates all micro-organisms colonizing an individual.
The composition of the normal flora is not identical in people all over the world. This provides a way to identify some organisms that belong to the normal flora but not to the autochthonous flora. For example, in a large part of the world, healthy adults excrete *Escherichia coli* strains, belonging to their normal bowel flora, that may kill their children and sicken many tourists. So the normal flora may contain real pathogens. Absence of these organisms in people in other parts of the world appears to be perfectly compatible with normal health, so these organisms do not belong to the autochthonous flora.
Dubos emphasized that the normal flora consists of high numbers of obligate anaerobic flora and much lower, erratic numbers of aerobic flora. He concluded that enterococci and Enterobacteriaceae do not belong to the autochthonous flora because "*..in all colonies of mice so far tested, the changes in population size of enterobacteria and enterococci (shortly after birth) suggest that these organisms cause an intestinal infection, but one from which the animals recover. In this light, the persistence of these organisms in the faces would correspond to a carrier state, or to a low grade continuous infectious process*" (2). Summarizing, in his opinion the protective flora is composed of (an unknown part of the) obligate anaerobic flora, and aerobic flora is potentially pathogenic.

The concept of colonization resistance

The term "colonization resistance" was introduced by van der Waaij, who presented further evidence that the obligate anaerobic flora of the bowel limits colonization of the bowel by exogenous aerobic flora (3), and also limits the faecal concentration of indigenous aerobic flora (4, 5). He emphasized the importance of sparing the flora that provides CR when using antimicrobial chemotherapy, presuming the availability of antimicrobials that did so (6). By this presumption, the fatalistic attitude that administration of antibiotics inevitably increases the risk of superinfections, changed into the hopeful expectation that this problem might be circumvented. If the

concept of CR is right, the risk of antibiotic-related superinfections will be eliminated by selecting antibiotics that do not disturb that part of the anaerobic flora that provides CR (CR flora). This would not be a problem, because most infections are caused by aerobic flora. Furthermore, it would be possible to prevent infections in compromised patients, by elimination of indigenous aerobic flora with antibiotics that do not disturb the CR-flora.
This concept challenges the conclusions that had been drawn from prophylaxis with penicillins and tetracyclines. Experience had taught that prophylactic administration of these antibiotics to compromised patients increases the risk of infections (7, 8, 9, 10). Therefore, prophylactic administration of antibiotics was strongly advised against by microbiologists, in general with rather limited success. Instead, the concept of CR predicts that it is possible to eliminate indigenous aerobic flora prophylactically, without increasing the risk of superinfections by resistant strains (selective decolonization).

Clinical observations supporting the concept

The concept of CR was based on data from experiments in animals. However, it cannot be taken for granted, that the influence of antimicrobial agents in man and mice is identical. Therefore, this issue has to be studied in man. Unfortunately, interpretation of data on the influence of antibiotics on CR in patients is rather complicated, because CR may be impaired by illness (11), old age (12), hospitalization (13) and stress of surgery (14). Still, it has been shown in patients that the risk of superinfections is not increased to the same extent by all antibiotics. For example, cefaclor (which was presumed to spare the CR) caused less oropharyngeal colonization by Enterobacteriaceae and yeasts and less superinfections than amoxycillin (which impairs CR) in patients treated for pulmonary infections (15). A clinical trial in neutropenic patients showed that therapy with a combination of aztreonam plus tobramycin, which had a minimal impact on the intestinal flora, was associated with fewer fungal infections than either aztreonam plus cloxacillin or moxalactam plus tobramycin, both of which more strongly disrupted the intestinal anaerobic microflora (16). These data were considered as indications that, in man also, disturbance of anaerobic flora

increases the risk of superinfections.
Much more supportive evidence for the concept came from prophylactic administration of antibiotics. When co-trimoxazole was given on a long-term basis to children with acute leukaemia, in order to prevent *Pneumocystis carinii* infections, an unexpected benefit was a decrease in the number of bacterial infections compared to the placebo group (17). Subsequently, successful prophylaxis of infections in neutropenic patients by long-term administration of co-trimoxazole or quinolones has been demonstrated in a large number of studies (18, 19). Proponents of the concept of CR ascribed the success of these prophylactic regimens to elimination of Gram-negative bacilli from the bowel without disturbing the CR-flora. Therefore, this method of prophylaxis was called "selective decontamination" (18). In the rest of this paper we will use the term "decolonization", because "decontamination" is associated with elimination of micro-organisms from inanimate objects.
Selective decolonization has also been applied successfully for prevention of infections in patients who are mechanically ventilated (20), and for prevention of Gram-negative bacilluria in patients with transurethral catheters (21).
It should be noted that these regimens were called "selective" because it was **assumed** that they did not impair CR in man, but this was based on extrapolation of data obtained in mice.

Definition of the concept of colonization resistance

Up to here, we have described "CR" as a philosophy, but that does not provide a suitable basis to test the concept. Validation of a theory requires verifiable hypotheses. To this purpose the concept had not been defined properly. On the contrary, much confusion has arisen because "CR" does not mean the same to every author at every time.
The term CR was introduced by van der Waaij in 1971, to indicate resistance against colonization by exogenous strains quantitatively. He defined it as the logarithm of the bacterial dose resulting in colonization for at least two weeks in 50% of the contaminated animals (3).
In 1974 van der Waaij reported that the faecal concentration of

enterococci and yeasts remained unchanged following elimination of Enterobacteriaceae from the bowel of mice and monkeys by administration of nalidixic acid. From this he concludes : "*This indicates that the fraction of the anaerobic microflora which is responsible for the CR of the digestive tract is not affected by the treatment*", and (in the discussion) " *Inasmuch as the concentration of enterococci remained unchanged during the treatment, it appears that the CR was unaffected. The fact that the C. albicans concentration remained low in the monkeys during treatment further supports the interpretation that nalidixic acid does not affect CR*" (5). In other words, increase in enterococci or yeasts would have been taken as evidence of impairment of CR. From this we concluded, that the limiting action of CR-flora on the faecal concentration of indigenous microflora had been included in the definition of CR by that time.

In 1986, however, van der Waaij again defines "the concept of CR" as "*the ability of the normal gut flora to combat colonization by extrinsic bacteria*" (22). The same definition is used by Nord (23). So, these authors do not include the influence on indigenous flora in the definition of CR. It is not clear whether this implies that an increase in the concentration of parts of the indigenous aerobic flora is not considered impairment of CR by these authors. However, Barza *et al.* are explicit at this point. They define CR as "*the resistance displayed by the normal host to the implantation of new strains (as opposed to overgrowth of already present strains) in the faecal microflora*" (24).

We think this is highly confusing. If the CR-flora is considered to have a limiting action on both exogenous flora and on indigenous flora, it is not logical to reserve the term CR for resistance against colonization by exogenous strains. We considered it more logical to interpret the term (microbial) CR as the limiting action (of autochthonous micro-organisms) on colonization of the body by potentially pathogenic micro-organisms, both exogenous and endogenous. The limiting action results in total exclusion from the bowel of most species of aerobic flora, and in a restricted concentration of some specially adapted aerobic species (indigenous aerobic flora). This was our approach from the first study onwards (chapter III).

So, in our interpretation, the concept of colonization resistance

consisted of the three following hypotheses (chapter III) :
1. Microbial CR against aerobic flora is provided exclusively by obligate anaerobic flora (2, 3).
2. Microbial CR limits colonization of the bowel by exogenous micro-organisms (2, 3).
3. Microbial CR limits the faecal concentration of indigenous aerobic micro-organisms (2, 4, 5).
In general, the data of our studies up to now have not necessitated major changes in this concept. As will be discussed however, indigenous Gram-negative bacilli may contribute to CR against exogenous Gram-negative bacilli if the CR-flora has been disturbed.

Study design for the classification of antimicrobial agents according to their influence on colonization resistance

1. Study subjects
The first data on the influence of antimicrobial agents came from experiments in animals, mostly mice.
Animal studies have been important for demonstration of the existence and of the potential clinical importance of CR. The first tentative classification of antimicrobial agents according to their influence on CR in man, was based on extrapolation of data obtained in mice (6). However, it is unlikely that the CR-flora is identical in mice and in man. Moreover, the dosage of an antimicrobial agent that has been used in animal experiments can not be reliably extrapolated to man. Accurate extrapolation is of utmost importance however, because it has been shown that the influence of penicillin on CR in man strongly depends on the dosage (10). Therefore, classification of antimicrobial agents according to their influence on CR has to be based on studies performed in man.
As argued before, patients are not ideal subjects for this kind of investigations, because the concentration of indigenous aerobic flora may be increased due to their disease. Moreover, it is difficult to obtain faeces from hospital patients regularly. Therefore, volunteers are to be preferred for studies on the influence of antimicrobial agents on CR.
In some studies the study subjects are called volunteers, but it appears that these volunteers are patients, so the results of these

studies have to be interpreted cautiously (25).

2. What should be measured ?

2.a. Anaerobic flora ?

Although we presume that microbial CR is provided by anaerobic flora, it is our opinion that analysis of anaerobic flora in faeces does not give useful information on the influence of antimicrobial agents on CR. Firstly, the composition of the CR-flora is unknown and most anaerobic species cannot be cultured selectively. Secondly, it is not certain that impairment of CR requires reduction of the concentration of anaerobic species involved in CR ; reduction of the production of inhibiting substances might be sufficient. So, although all data on the influence of antimicrobial agents on the faecal flora are of scientific interest, determination of the concentration of the few cultivatable anaerobic species does not contribute to knowledge concerning the influence of antimicrobial agents on CR.

Some authors rely, at least partially, on anaerobic flora as an indicator of impairment of CR (24, 25, 26). This may lead to wrong conclusions.

2.b. Total aerobic flora ?

Some investigators use the total concentration of aerobic flora as an indicator of the influence of antimicrobial agents on CR (26). This gives no reliable information however, because the total concentration remains unchanged if the dominant group of aerobic flora is not affected. On the other hand, a decrease of the faecal concentration of the dominant group of aerobic flora may cause a decrease of the total concentration of aerobic flora, even if the faecal concentration of another group of aerobic flora is increased. For example, significant increase in the faecal concentration of yeasts indicated impairment of CR in three volunteers following pefloxacin 400 mg twice daily, but the total faecal concentration of aerobic flora decreased due to reduction of the concentration of Gram-negative bacilli and aerobic Gram-positive cocci (chapter XI).

2.c. Groups of aerobic flora

Impairment of CR can be demonstrated by an increase in the faecal concentration of Gram-negative bacilli, aerobic Gram-positive cocci or yeasts, and by facilitation of colonization of the bowel by other Gram-negative bacilli than *E. coli*. Of course, an increase in aerobic flora following impairment of CR will occur only if resistant species are present in the bowel before or are ingested with food during

administration of the antimicrobial agent. Therefore, the concentration of Gram-negative bacilli and aerobic Gram-positive cocci may give false negative results. However, yeasts are not susceptible to antibacterial agents. Therefore, yeasts are a useful endogenous indicator of impairment of CR if the faecal concentration of antibacterial agents is high.

Furthermore, it should be noted that detection of an increase in the occurrence of low concentrations of Gram-negative bacilli other than *E. coli*, following antimicrobial agents that decrease the faecal concentration of *E. coli*, does not necessarily prove an actual increase in occurrence of those strains, because elimination of *E. coli* makes it much easier to detect low level concentrations of other Gram-negative bacilli. This problem can be reduced by using selective culture media, apart from the usual ones. If the antimicrobial agent under investigation is added to the culture medium for Gram-negative bacilli, this improves the chance of detection of low-level colonization by secondary Gram-negative bacilli during the pre-treatment period.

Increase of enterococci has been misinterpreted in some studies. Since it was assumed that cefotaxime spares CR, cefotaxime was excluded as a possible cause of an observed increase of the faecal concentration of enterococci. As a consequence, the increase of enterococci was tentatively explained as a result of selective decontamination (16, 27, 28). However, selective elimination of *E. coli* does not cause an increase in the faecal concentration of enterococci (chapter IX), but cefotaxime does (chapter VII).

2.d. Challenge-strains.

Impairment of CR will go unnoticed if resistant micro-organisms are not present. Therefore we advice to use challenge strains. So far, our experience is limited to challenge with Gram-negative bacilli. The ideal challenge strain is a strain that has been acquired from surveillance cultures of a patient treated prophylactically with the antimicrobial agent one wants to investigate, provided that this strain did not cause problems to the patient. Moreover, the strain should be susceptible to non-toxic antibiotics.

The challenge strain should be administered twice, once during the pretreatment period and once during the treatment period. If administration of the antimicrobial agent facilitates colonization by the challenge strain, this indicates impairment of CR.

It should be emphasized that the challenge strain has to be resistant to the active antimicrobial concentration in the bowel. This may deviate strongly from the levels achievable in blood. For example, erythromycin is not effective for treatment of infections with Gram-negative bacilli. Yet it eliminates Gram-negative bacilli from faeces (chapter III).
It is a problem that the methodology for analysis of the active faecal concentration of antimicrobial agents has not been developed yet. We have found indications that the active faecal concentration of quinolones is about equal to the diffusible faecal concentration (chapter XI). However, these data are preliminary and it is not known whether similar data will be found with other groups of antimicrobial agents. As long as this problem has not been solved, failure of colonization of the bowel by a challenge strain suggests absence of impairment of CR only when the strain does grow out when an antimicrobial agent impairing CR is added to the agent under investigation (chapter IX).
Apart from technical problems, emotional problems have hampered the use of challenge strains. Many investigators are afraid to administer bacteria to volunteers. In fact however, the risk of administration of a challenge strain (with the specifications described before) does not exceed the risk of ingesting Gram-negative bacilli contained in normal food. On the contrary, the risk of the challenge strain is lower because it is known before that the challenge strain is susceptible to non-toxic antibiotics.
The paper of Barza *et al.* (24) is frequently cited as evidence that the importance of anaerobic flora for CR is doubtful. In that study however, challenge strains were used which were susceptible to the antibiotics under investigation (for ethical reasons). Because the challenge strains did of course not succeed to colonize when administered during antibiotic treatment, the challenge was repeated after treatment, and the duration of colonization was recorded. However, this is not a reliable method either, because CR may be restored soon after the end of treatment, as we have seen with pefloxacin (chapter XI).

3. Statistical analysis of the data

In the usual study design, the influence of antimicrobial agents on faecal flora is investigated by studying its influence on the median faecal concentration in a group of volunteers (25). However, the

antimicrobial susceptibility of the CR-flora may differ between volunteers. Therefore, the influence on CR is not necessarily the same in all volunteers. For example we found no impairment of CR in three of 11 volunteers following amoxycillin (chapter VI) and in three of six volunteers following pefloxacin (chapter XI). Obviously, the chance of obtaining a significant increase in the median faecal concentration of aerobic flora in a group of volunteers decreases with the proportion of volunteers in whom CR is not impaired. This may explain why we did not demonstrate impairment of CR following pefloxacin when the data were analyzed for the group of volunteers (chapter X). We conclude that the influence of antimicrobial agents on CR has to be analyzed in each volunteer separately. The normal fluctuation of the faecal concentration of Gram-negative bacilli is such that a tenfold change will be demonstrated with statistical significance if ten pre-treatment samples and ten treatment samples are used (chapter VI).
We wish to emphasize that each volunteer should be his or her own control. In the paper of Barza (24), the duration of colonization by challenge strains administered after treatment with antibiotics was compared to the duration of colonization in four volunteers who served as a control. This is not a reliable study design, because susceptibility to challenge strains depends on many factors and may show large differences between volunteers (chapter VI).

Validation of the concept of colonization resistance

At the start of our studies we had little doubt concerning the rightness of that concept. Our only intention was to separate the safe drugs from the dangerous ones, expecting that we would add evidence of the rightness of the concept at the same time.
However, we got into problems almost immediately. We observed an increase in the number of samples with Gram-negative bacilli other than *E. coli* (secondary Gram-negative bacilli), following agents that did not increase the <u>total</u> faecal concentration of Gram-negative bacilli, of enterococci or of yeasts. So according to our hypothesis, the CR-flora was not impaired. The most simple explanation would be that increase of secondary colonization was just an artefact, due to the fact that it is easier to detect low level

concentration of secondary Gram-negative bacilli following a decrease of the faecal concentration of *E. coli*. Though this could not be excluded, we did not consider it a very likely explanation. Therefore, we introduced the hypothesis of "substitution colonization", which states that low-level secondary colonization is facilitated by a decrease of the faecal concentration of *E. coli* itself, and does not proof impairment of the CR-flora.
Re-evaluating the data of the first two studies (chapters III and IV), we realized ourselves that both the hypotheses and our study design were not satisfactory. Obviously, outgrowth of aerobic flora will occur only if resistant species are present, and the antimicrobial susceptibility of the CR-flora may differ between volunteers. Moreover, the hypothesis of substitution colonization necessitated a change in the hypothesis that CR is provided exclusively by anaerobic flora, and the original hypotheses (page 163) did not state clearly whether impairment of CR is expected to cause an increase in all three groups of aerobic flora. Therefore, we changed our study design as discussed in the preceding section, and reformulated our hypotheses as follows :
1. The total faecal concentration of Gram-negative bacilli, of aerobic Gram-positive cocci and of yeasts is limited exclusively by an anaerobic flora that provides CR.
2. Impairment of the anaerobic flora that provides CR will cause an increase in the faecal concentration of Gram-negative bacilli as well as of aerobic Gram-positive cocci and of yeasts, if resistant strains are present.
3. Selective elimination of *E. coli* facilitates low level colonization of the bowel by other Gram-negative bacilli (substitution colonization).
4. Disturbance of the anaerobic flora that provides CR will result in high-level colonization by resistant challenge strains.

We will now discuss the results of the validation of these hypotheses.

ad 1. *The faecal concentration of Gram-negative bacilli, of aerobic Gram-positive cocci and of yeasts is limited exclusively by an anaerobic flora that provides CR.*
Up to now, we have not obtained data that speak against this

hypothesis. Elimination of indigenous Gram-negative bacilli with 20 mg pefloxacin daily, did not cause an increase in the faecal concentration of enterococci or yeasts (chapter IX). So it would appear that Gram-negative bacilli do not suppress enterococci or yeasts (at least if the CR-flora is not disturbed). Several volunteers with normal CR were not colonized by yeasts. So yeasts are also not important for CR. The role of enterococci is more difficult to ascertain, because they colonize almost all people and because agents for selective decolonization of enterococci are not available. However, in one of our volunteers CR was maintained in the pre-treatment period of the amoxycillin-study, although enterococci were not detectable in eight of ten samples (chapter VI). This makes it unlikely that the contribution of enterococci is of major importance. Further, we observed a simultaneous increase in the concentration of Gram-negative bacilli, of aerobic Gram-positive cocci and of yeasts following amoxycillin (in one volunteer who appeared to have acquired resistant enterococci, chapter VI), clindamycin (chapter IX) and cefotaxime (chapter VII). The same had been described before following cefoxitin (30). Simultaneous increase of the three groups of aerobic flora indicates that they have no major mutual inhibitory action.

Conclusion : Increase in the faecal concentration of Gram-negative bacilli, aerobic Gram-positive cocci or yeasts indicates disturbance of an anaerobic flora that provides CR.

ad 2. *Impairment of the anaerobic flora that provides CR causes an increase in all three groups of indigenous aerobic flora, if resistant species are present.*

This hypothesis states that the limiting action of species of the anaerobic flora on aerobic flora is not selective to subgroups of aerobic flora. We have just mentioned some observations demonstrating that a simultaneous increase of the three groups of aerobic flora is possible. However in four of five volunteers in whom a significant increase in the faecal concentration of enterococci and yeasts indicated disturbance of the CR-flora following cefotaxime, the faecal concentration of Gram-negative bacilli did not increase above the level in the pre-treatment period, although the bowel was colonized with a highly resistant challenge strain (chapter VII). Moreover, in one volunteer in whom the faecal concentration of

Gram-negative bacilli and enterococci increased strongly following clindamycin, outgrowth of yeasts did not occur (chapter IX).
So, it appears that disturbance of the CR-flora is not always followed by overgrowth of all groups of aerobic flora. Possibly, CR-flora consists of several species that do not inhibit all three groups of aerobic flora to the same extent. It is also possible that factors other than CR-flora (for example antibodies in mucous secretions or absence of essential nutrients), do not impede outgrowth of specific aerobic species to the same extent in all volunteers. This might be investigated by impairing CR in such volunteers with other antimicrobial agents, but such studies have not been performed.
Conclusion : Absence of an increase in the concentration of one of the three groups of aerobic flora does not disprove impairment of the CR-flora.

ad 3. *Selective elimination of Escherichia coli facilitates low-level colonization of the bowel by other Gram-negative bacilli (substitution colonization).*
Contrary to our expectation, selective elimination of indigenous Gram-negative bacilli did not facilitate low-level colonization by a resistant challenge strain (chapter IX). Daily administration of 20 mg pefloxacin eliminated Gram-negative bacilli from faeces, but did neither cause an increase in the faecal concentration of enterococci or yeasts, nor did it facilitate colonization of the bowel by a highly resistant strain of *Klebsiella pneumoniae*, whereas clindamycin enabled high-level colonization by this strain in all volunteers. Although this study does not exclude entirely that the phenomenon of substitution colonization may exist, it is unlikely that it occurs very easily. Therefore, it is our present view that the increase of secondary colonization following co-trimoxazole, doxycycline and roxithromycin which we observed in previous studies (chapters III and IV), should be regarded as suggestive evidence that these agents impair the CR-flora. The fact that the faecal concentration of secondary Gram-negative bacilli remained below the pre-treatment value of *E. coli* following those antimicrobial agents, is probably due to the fact that the active faecal concentration of antimicrobial agents was too low to cause complete elimination and too high to allow overgrowth of these secondary Gram-negative bacilli. This issue should be investigated by comparing the MIC of colonizing

Gram-negative bacilli with the faecal concentration of the agent under investigation.
Conclusion : Increase of low-level secondary colonization should be regarded as suggestive evidence of disturbance of the anaerobic flora that provides CR.

ad 4. *Disturbance of the anaerobic flora that provides CR results in high-level colonization by challenge strains.*
Although disturbance of the anaerobic CR-flora may enable high-level colonization of the bowel by resistant challenge strains, false negative results are possible. It appears that colonization of the bowel by exogenous Gram-negative bacilli following disturbance of the CR-flora may be impeded by indigenous Gram-negative bacilli. We have found definite examples of mutual competition between Gram-negative bacilli. When microbial CR against indigenous flora was impaired (as indicated by an increase in one of the three groups of aerobic flora), colonization of the bowel by a resistant challenge strain occurred more reliably if other Gram-negative bacilli were suppressed (chapters VII, IX and XI), and could fail if indigenous Gram-negative bacilli were resistant and were growing out (chapters VI and VIII). This is in agreement with the observation in mice, that *E. coli* decreased the faecal concentration of *Shigella flexneri*, if the CR-flora was impaired (29, chapter II).
Conclusion : Failure of challenge strains of resistant Gram-negative bacilli to colonize the bowel does not disprove disturbance of the anaerobic flora that provides CR

In summary, our studies give further support to the hypothesis that the microbial CR is provided by autochthonous anaerobic flora, and that it is possible to eliminate aerobic flora from the bowel without increasing the risk of superinfections. However, impairment of CR-flora does not invariably cause an increase of all three groups of aerobic flora. Moreover, if CR-flora is impaired, indigenous Gram-negative bacilli may contribute to the CR against exogenous Gram-negative bacilli.

It should be emphasized that the influence on CR is of no importance when all potentially pathogenic micro-organisms are killed by the antimicrobial agents. So, the influence on the CR-flora only modifies the influence of antimicrobial agents on the faecal concentrations of surviving potentially pathogenic micro-organisms.
The influence of antimicrobial agents on the aerobic flora of the bowel depends on 1) the active concentration of antimicrobial agents in the bowel, 2) the susceptibility of aerobic micro-organisms and 3) the susceptibility of the CR-flora.
It should be noted that " antibiotic-susceptibility of CR-flora" includes impairment of the production of inhibiting substances, apart from bactericidal or bacteriostatic action. The theoretical possibilities are as follows (Figure).
Possibility 1 : Both aerobic flora and the CR-flora are not affected.
We have not yet found an example of possibility 1. Agents used for the treatment of lower urinary tract infections have the best chance to meet the requirements. If absorption is complete and excretion in urine is rapid, such agents may have bowel concentrations and tissue levels below the MIC of any indigenous micro-organism. However, contrary to our expectations, cephradine 500 mg twice daily did not fulfil these requirements (chapter VIII).
Possibility 2 : (Part of the) aerobic flora is affected and the CR-flora is not affected (selective decolonization).
The only example of this possibility we have demonstrated so far is pefloxacin 20 mg once daily (chapter IX). This is not a therapeutic dosage, but the experiment was devised to investigate whether selective decolonization is possible at all.
Possibility 3 : (Part of the) aerobic flora is not affected and the CR-flora is impaired.
Examples of possibility 3 are amoxycillin, cefotaxime and clindamycin (chapter VI, VII and IX). These agents impair CR in most or in all volunteers. Their spectrum of activity against aerobic flora is not the same, however, causing differences in the influence of these agents on the aerobic flora of the bowel :
- Clindamycin causes an increase in the faecal concentration of Gram-negative bacilli, aerobic Gram-positive cocci and yeasts.

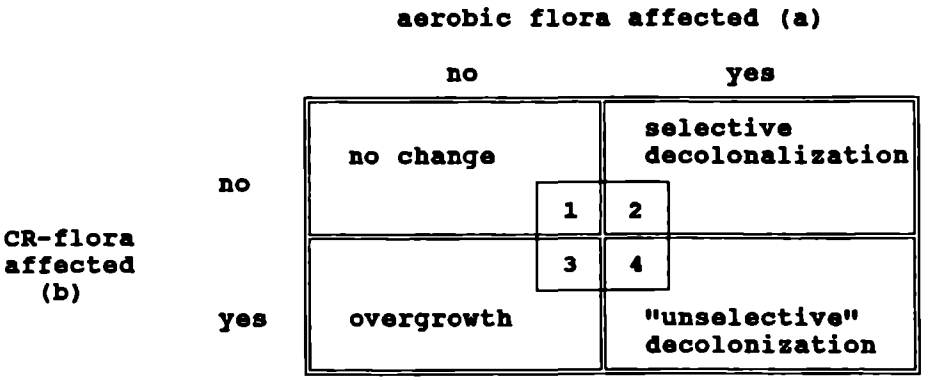

Figure. Influence of antimicrobial agents on the concentration of aerobic flora in the bowel.
(a) : Active faecal concentration > MIC aerobic micro-organisms.
(b) : Active faecal concentration > MIC CR-flora or high enough to decrease production of inhibiting substances.

- Amoxycillin causes an increase in the faecal concentration of Gram-negative bacilli and yeasts, but a decrease in the faecal concentration of enterococci. Overgrowth of enterococci may occur, however, if resistant enterococci are acquired during therapy.
- Cefotaxime causes an increase in the faecal concentration of enterococci and yeasts, but resistant Gram-negative bacilli are not prevalent. Therefore the faecal concentration of indigenous Gram-negative bacilli will generally decrease. However, if resistant Gram-negative bacilli are acquired, overgrowth may occur.

Possibility 4 : Both aerobic flora and the CR-flora are affected ("unselective decontamination").

Examples of possibility 4 are norfloxacin 400 mg three times a day and pefloxacin 400 mg three times a day (31). At this dosage, Enterobacteriaceae were eliminated from faeces in all five patients and enterococci in four of five patients. However, 400 mg two times a day already disturbs the CR-flora, causing outgrowth of yeasts (chapter XI). In the study of Giuliano this was not observed, because the patients were protected by simultaneous treatment with oral amphotericin B (31). Each regimen for decolonization that contains one or more components that impair CR falls into category 4, for example cefotaxime-containing regimens (chapter VII).

Discussion

At the start of our studies, we intended to validate and expand the "green list" of CR-sparing antimicrobial agents (6) and to improve the safety of antibiotic therapy in our hospital by reserving antimicrobial agents that impair CR for treatment of infections for which no CR-sparing agent was available. We improved the study design for investigation of the influence of antimicrobial agents on CR and we could demonstrate that it is possible to eliminate Gram-negative bacilli from faeces without impairing microbial CR. However, our studies unmasked most "safe" antibiotics and did not add new ones.

So, although it remains our ideal to select antimicrobial agents for therapy that do not disturb microbial CR, most (if not all) presently available antimicrobial agents do not fulfil this criterion. Therefore, it is important that the pharmaceutical industry focuses on the development of antimicrobial agents with a large difference of

activity against aerobic potentially pathogenic micro-organisms on one hand, and against known anaerobic species on the other hand.
In the mean time, we will give preference to the second best group of antimicrobial agents, *id est* those agents that achieve sufficiently high active concentrations in the bowel to inhibit outgrowth of aerobic bacteria. It should be noted that in this way *Candida* spp. will not be covered, and that problems will occur if resistant aerobic bacteria or *Clostridium difficile* are acquired. Unexpectedly, this problem is illustrated by the development of bloody diarrhoea in one of our volunteers during cefotaxime and by the frequent occurrence of *Candida*-vaginitis in female volunteers in our studies. Bloody diarrhoea following challenge with *Enterobacter cloacae* has also been observed in a study with amoxycillin (32). So, contrary to our original expectations, it appeared that overgrowth of resistant micro-organisms due to disturbance of CR may cause infections in healthy volunteers with (presumably) normal infection resistance.
In females between 20 and 50 years old, *Candida* vaginitis probably is the most frequently occurring superinfection. So, female patients and volunteers should be warned for this problem, as well as for the risk of failure of oral anticonception following antimicrobial agents (chapter V). Even although all or most available antibacterial agents increase the risk of superinfection with *Candida* spp., those agents that rarely allow overgrowth by resistant bacteria remain preferable to those that cause this effect in the majority of patients. In this regard, co-trimoxazole, doxycycline and quinolones are preferable to penicillins.
The importance of "selectivity" of antimicrobial agents used for prophylaxis has been overestimated. This idea arose from the observation that "selective decontamination" was more effective in the prevention of infections in leukopenic patients than prophylaxis by regimens that were known to impair CR (18). The idea was strengthened by the success of "selective decontamination" in patients who are mechanically ventilated. As Gurwith rightly noted however, the absolute necessity of selectivity can easily be refuted by the observation that addition of vancomycin (which impairs CR) to co-trimoxazole (which was used for selective decontamination in granulocytopenic patients) did not abolish the prophylactic efficacy of co-trimoxazole, but actually increased it (33). A similar argumentation applies to "selective decontamination" in patients who

are mechanically ventilated. Cefotaxime is included in most of these regimens (20), because it improved the results when added to oral non absorbable agents (34). We could show, however, that cefotaxime impairs CR (chapter VII).
So, effective concentrations against prevailing potentially pathogenic micro-organisms are more important than sparing of the CR-flora. As selectivity is not an absolute requirement and as most regimens used for "selective decontamination" are not selective, we recommend to change the term "selective decontamination" into "prophylactic decolonization". It is striking that, in this way, we return again to the terminology that has been used in one of the first publications of the clinical application of prophylactic decolonization in leukopenic patients : "partial antimicrobial decontamination" (35). It now appears that "partial" is much more appropriate than "selective" for the regimens that have been used so far.
Presently, quinolones are the safest antimicrobials for prophylactic decolonization and for therapy, as far as the risk of superinfections is concerned. Aerobic bacteria resistant to the diffusible faecal concentration of norfloxacin and pefloxacin do not exist. Disturbance of CR following pefloxacin occurred in half of our volunteers, but was reversed before the faecal concentration of pefloxacin dropped far enough to allow recolonization by Gram-negative bacilli acquired with food. The same will probably apply to norfloxacin. In standard dosage (400 mg twice daily) the diffusible faecal concentration of norfloxacin is about 400 mg/l (not published). Norfloxacin was used successfully as the basis of regimens for prophylactic decolonization of the bowel in patients on mechanical ventilation (36, 37, 38), in neutropenic patients (39), and in patients with transurethral catheterization (21). An important advantage of norfloxacin is that it is not only very effective, but also very cheap.
For treatment of systemic infections with Gram-negative bacilli, third generation quinolones are preferable to aminoglycosides and to cephalosporins like cefuroxime, cefotaxime and ceftazidime. The ratio between tissue levels and MIC's is at least as good, there is little inoculum effect on the MIC, enzymatic inactivation in faeces has not been observed and we have never succeeded in obtaining Gram-negative bacilli resistant to the diffusible faecal concentration of pefloxacin. Furthermore, a major advantage of quinolones is that oral administration is possible.

The weak spot of quinolones is their minor activity against streptococci and enterococci at achievable tissue levels. If these organisms have to be covered at the same time as Gram-negative bacilli, combination of quinolones with a penicillin is preferable to combination with third generation cephalosporins. In this kind of combinations, quinolones may prevent the overgrowth with Gram-negative bacilli, which is associated with administration of penicillins alone.

Conclusion

Antimicrobial agents may cause superinfections by overgrowth of resistant micro-organisms as a result of disturbance of (part of the) autochthonous anaerobic microflora of the digestive tract. Therefore, it would be desirable to select antimicrobial agents that do not cause this unfavourable effect.

The influence of antimicrobial agents on the aerobic flora of the bowel has to be investigated in healthy volunteers, because extrapolation of animal data to man is not reliable and because in patients an increase in the aerobic flora may be caused by their disease.

The data have to be analyzed for each volunteer individually, because the susceptibility of the CR-flora to antimicrobial agents is not necessarily the same in all volunteers. The normal fluctuation of the concentration of aerobic flora is such, that a tenfold change in the faecal concentration will be demonstrated with statistical significance if ten pre-treatment samples and ten treatment samples are used.

In patients with normal CR, the faecal concentration of aerobic flora is not limited by Gram-negative bacilli, aerobic Gram-positive cocci or yeasts. Thus, the hypothesis still remains unaffected, that microbial CR is provided by obligate anaerobic flora. Yet, the faecal concentration of anaerobic species does **not** give information on the microbial CR, because the composition of the CR-flora is unknown, and because reduction of the production of inhibiting substances by the CR-flora might occur although the concentration of the CR-flora remains unchanged.

When CR is impaired, competition between Gram-negative bacilli

may occur. Therefore, resistant challenge strains will not always reveal impairment of CR.
Disturbance of the CR-flora may cause an increase in the concentration of Gram-negative bacilli as well as of aerobic Gram-positive cocci and of yeasts. Of course, a prerequisite is the presence of strains resistant to the active concentration of the antimicrobial agent. It should be noted that the active concentration of antimicrobial agents may be much higher in the bowel than in plasma. For example, the active faecal concentration of pefloxacin and norfloxacin is so high, that impairment of CR by these agents will not be followed by an increase in the faecal concentration of aerobic bacteria, because sufficiently resistant aerobic bacteria do not exist or are extremely rare. Yeasts are not susceptible to antibacterial agents. Therefore the faecal concentration of yeasts is a useful endogenous indicator of CR when the faecal concentration of antibacterial agents is high.
Although impairment of microbial CR removes a major impediment to outgrowth of aerobic flora, impairment of microbial CR does not always result in an increase in the faecal concentration of all three groups of aerobic flora. It is not known whether this is explained by selectivity of the limiting action of some anaerobic species to special parts of the indigenous aerobic flora, or by interindividual differences in other inhibiting factors, for example secretion of antibodies in mucous fluid or absence of essential nutrients.
Despite impairing CR, some antimicrobial agents do not cause bacterial overgrowth because their active antimicrobial concentration in the bowel is higher than the MIC of prevailing aerobic bacteria. These agents will be unmasked when resistant challenge strains are administered, or when the concentration of yeasts is studied. For the same reason, these agents increase the risk of superinfections with yeasts and they will cause problems when resistant bacteria are encountered.
As the indigenous aerobic flora does not contribute to normal CR, it is theoretically possible to treat aerobic infections with antimicrobial agents that do not increase the risk of superinfections. Unfortunately, it has not been shown for any antimicrobial agent that it spares the microbial CR in all volunteers when administered in normal therapeutic dosage. Selective decolonization is possible however by administration of a low dosage of quinolones.

The common belief that the risk of superinfections may be reduced by avoiding broad-spectrum agents is not right. On the contrary, as long as we do not dispose of antimicrobial agents that spare the CR-flora (in therapeutic dosage), agents with the broadest spectrum of activity against aerobic flora and high active concentration in the bowel will cause the lowest risk of bacterial superinfections. In this regard, quinolones are the preferable agents for prophylaxis and for therapy of infections by Gram-negative bacilli. In patients with strongly decreased resistance to infection however, oral amphotericin should be added for prevention of fungal infections.

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According to the concept of colonization resistance (CR), (part of) the autochthonous anaerobic flora limits the concentration of potentially pathogenic micro-organisms in the digestive tract. If this concept is right, occurrence of superinfections caused by antimicrobial agents (AMA) may be avoided by using AMA which do not disturb the anaerobic flora that provides CR (CR-flora). If the active concentration of those AMA in the digestive tract is higher than the minimal inhibitory concentration (MIC) of normally occurring potentially pathogenic micro-organisms, they can be used safely for selective decolonization.
It was our aim to investigate which AMA are preferable (from this point of view) for prophylaxis and for therapy of infections.

Chapter I explains why we started these investigations and briefly discusses results of the studies and considerations that lead to subsequent investigations.

Chapter II reviews the literature on this subject.

Chapters III and IV describe the influence on CR of amoxycillin, cefaclor, co-trimoxazole, doxycycline, erythromycin, phenethicillin and roxithromycin. These studies demonstrated impairment of CR following amoxycillin and phenethicillin. The faecal concentration of Gram-negative bacilli, enterococci or yeasts did not increase following the other antimicrobials, but low-level colonization with Gram-negative bacilli other than *E. coli* (secondary colonization) was seen more often, especially if the faecal concentration of *E. coli* had been reduced. The faecal concentration of secondary Gram-negative bacilli did not increase above the concentration of *E. coli* in the pre-treatment period, however, as occurred following amoxycillin and phenethicillin. It seemed as if a normal concentration of *E. coli* was necessary to prevent secondary colonization, and as if a decrease in the concentration of *E. coli* allowed low-level colonization with secondary Gram-negative bacilli ("substitution colonization"). Therefore, we felt that increase of low-level secondary colonization could not be regarded as a definite proof of disturbance

of the CR-flora. However, in chapter IX we found that elimination of *E. coli* with a low dose of pefloxacin did not facilitate colonization of the bowel by a highly resistant challenge strain of *Klebsiella pneumoniae* (secondary colonization), whereas this strain colonized the bowel in high concentration when clindamycin was added. Therefore, we now think that increase of secondary colonization is suggestive evidence of disturbance of the CR-flora.

Chapter V describes the influence of clindamycin, dicloxacillin, norfloxacin and minocycline on the faecal concentration of urobilinogen. We had come to the conclusion that challenge with resistant strains is necessary to show impairment of CR following AMA that eliminate normally occurring Gram-negative bacilli, but we were afraid to do so and looked for an alternative method.
It had been shown that strong impairment of CR could be shown by an increase in the faecal concentration of bèta-aspartylglycine, but this method was not sensitive enough to detect a small impairment of CR. In the mean time, it had been shown that urobilinogen is absent from faeces of germ-free animals and is absent or strongly reduced in faeces from animals treated with AMA that strongly reduce CR. AMA that were presumed not to impair CR, had no demonstrable effect on the faecal concentration of urobilinogen in animals. Therefore, we wanted to know whether it would be possible to use faecal urobilinogen as an indicator of the influence of AMA on the CR in man. Our study shows that the faecal concentration of urobilinogen was significantly reduced following clindamycin and dicloxacillin. These agents strongly reduce CR. Norfloxacin and minocycline, which were presumed to spare CR, also reduced the faecal concentration of urobilinogen, but not significantly. We concluded that decrease of the faecal concentration of urobilinogen was caused by inhibition of the anaerobic flora of the bowel, and was possibly useful for investigating the influence of AMA on CR.
Later studies showed, however, that minocycline impairs CR in part of the volunteers. The same applies to pefloxacin, which is comparable to norfloxacin. Hence the difference between clindamycin and dicloxacillin at one hand, and norfloxacin and minocycline at the other hand was caused by a difference in the degree to which the CR was impaired. So, the urobilinogen-method is also not sufficiently sensitive to demonstrate minor impairment of CR. Since

decrease of the faecal concentration of urobilinogen by AMA is caused by inhibition of metabolic activity of the anaerobic flora that deconjugates bilirubin-glucuronides, it is possible that such AMA disrupt enterohepatic cycling of substances which are deconjugated by microbial glucuronidases after excretion with bile, for example oestrogens. Therefore, administration of antibiotics that inhibit anaerobic flora might cause failure of oral anticonception.

Chapters VI, VII, VIII, IX and XI describe the influence on CR of amoxycillin, cefotaxime, cephradine, clindamycin and pefloxacin. The studies in chapters III and IV were performed according to the classical study design. However, this study design has major shortcomings. Therefore, the following adaptations were made.
1. Analysis in volunteers individually, instead of analyzing the data for the group of volunteers. From the normal fluctuation of the concentration of aerobic flora it was calculated that a tenfold change in the faecal concentration of aerobic flora would be detected with statistical significance with ten pre-treatment and ten treatment samples. This way, each volunteer served as its own control and administration of "control" AMA was not necessary, shortening the experiments considerably.
2. Administration of challenge strains, resistant to the active faecal concentration in the bowel. In this way, impairment of CR would not be missed by lack of resistant micro-organisms.
3. Use of selective culture media, in order to improve the chance of detecting secondary Enterobacteriaceae in the presence of high concentrations of *E. coli*.

Using this improved study design we investigated the influence of AMA on the faecal concentration of (1) the predominant Gram-negative bacilli, (2) of secondary Gram-negative bacilli occurring spontaneously or (3) administered intentionally and of (4) aerobic Gram-positive cocci and (5) yeasts.
When CR is impaired, all five indicators of impairment of CR may become positive. However, it appears that impairment of CR may allow competition among different species of Gram-negative bacilli. Therefore indicators 2 and 3 may remain negative if the indigenous *E. coli* is not susceptible to the AMA under investigation, and rapidly occupies the "space" that has come available due to

impairment of CR.
From this it follows that resistant challenge strains are not always the most sensitive indicator for impairment of CR, but only if the challenge strain has no competition from other Gram-negative bacilli.
Indicators 1, 4 and 5 may all become positive when CR is impaired. However, it was shown that this is not necessarily so. In one of five volunteers the concentration of yeasts did not rise following clindamycin (despite increase of the concentration of enterococci and yeasts), and in four of five volunteers in which the CR was impaired following cefotaxime (as shown by increase of enterococci and yeasts), the concentration of resistant Gram-negative bacilli did not increase above the pre-treatment level of Gram-negative bacilli. This points to the possibility of a certain selectivity of different species of the CR-flora as regards their inhibitory action on groups or species of aerobic flora of the bowel.

Contrary to our expectations, all agents investigated appeared to impair CR, although not to the same degree and not in all volunteers. *Candida* appeared to be a very reliable indicator of impairment of CR. *Candida* is carried by most volunteers and is resistant to antibacterial agents.
Following cefotaxime and pefloxacin, demonstration of impairment of CR against Gram-negative bacilli is possible only by administration of resistant challenge strains. Although we did not succeed in obtaining Gram-negative bacilli sufficiently resistant to the concentration of pefloxacin in the bowel during treatment, we could demonstrate impairment of CR against Gram-negative bacilli by administration of the challenge strain after the treatment-period in two of the three volunteers in whom impairment of CR was indicated by a significant increase in the concentration of yeasts. It should be noted, that this method is possible only if impairment of CR lasts longer than the active concentration in the bowel.

Chapter IX investigates the contribution of indigenous Gram-negative bacilli, especially *E. coli* , on the CR. We had found the active faecal concentration of quinolones to be about equal to the diffusible faecal concentration. The MIC for pefloxacin of most Gram-negative bacilli is below 4 mg/l. We calculated that this

concentration would be achieved in faeces with 20 mg pefloxacin orally once daily ; 2.5% of the normal daily dosage used for therapy. This dosage eliminated the Gram-negative bacilli from faeces, without causing a change in the faecal concentration of enterococci or yeasts, and without causing facilitation of colonization of the bowel by a highly resistant challenge strain of *Klebsiella pneumoniae*. This shows that indigenous Gram-negative bacilli do not contribute to CR. Therefore, selective decolonization of the bowel from Gram-negative bacilli, *id est* decolonization without disturbing the CR-flora, is possible.

Chapter X describes the influence of parenteral administration of pefloxacin on the faecal concentration of indigenous Gram-negative bacilli and of a highly resistant challenge strain. Indigenous Gram-negative bacilli rapidly disappeared from faeces and the challenge strain did not succeed in colonizing the bowel. Although this was the most resistant strain of Gram-negative bacilli we could obtain, the MIC of this strain (56 mg/l) was lower than the diffusible faecal concentration of pefloxacin (160 mg/l). Therefore this study did not prove that decolonization with pefloxacin 800 mg daily is selective. Moreover, the study design was not suitable to use the faecal concentration of yeasts as an indicator of the influence on CR in individual volunteers, because the study period was too short. Nevertheless, it was shown that parenteral administration of pefloxacin is highly effective in eliminating indigenous Gram-negative bacilli from faeces, and for prevention of new colonization by very resistant exogenous bacteria. This enables parenteral administration of pefloxacin for prophylactic decolonization in patients who can not be treated orally, for example because of vomiting.

Chapter XII discusses the findings of our studies and the implications for antimicrobial therapy. It is concluded that these studies give further evidence that the CR-flora consists exclusively of anaerobic bacteria. Therefore, it is theoretically possible to treat infections by aerobic micro-organisms without a risk of superinfections. However, most or all AMA presently available impair this flora in at least part of the population. Nevertheless, effective decolonization is possible at the present time already, by designing

regimens that achieve active concentrations of AMA higher than the MIC of known aerobic micro-organisms. AMA achieving active concentrations in the bowel higher than the MIC of prevailing aerobic bacteria cause a lower risk of bacterial superinfections, than AMA for which the concentration in the bowel is below the MIC of prevailing aerobic bacteria. In this regard, clindamycin and penicillins are the most dangerous AMA and quinolones are the safest available agents. However, the search for agents that do not impair CR should be continued.

SAMENVATTING

Volgens het concept van de kolonisatieweerstand (KW) beperkt (een deel van de) autochtone anaërobe flora de concentratie van potentieel pathogene micro-organismen in het spijsverteringskanaal. Als dit concept juist is, kunnen superinfecties ten gevolge van antimicrobiële middelen (AMM) worden vermeden door AMM te gebruiken die de KW-flora niet aantasten. Als bovendien de concentratie van deze middelen in het spijsverteringskanaal hoger is dan de minimale remmings-concentratie (MRC) van normaal voorkomende potentieel pathogene micro-organismen, dan is hiermee langdurige preventie van infecties mogelijk (selectieve dekolonisatie).
Het was onze doelstelling te onderzoeken welke AMM vanuit deze optiek de voorkeur verdienen voor preventie en therapie van infecties.

In **Hoofdstuk I** wordt beschreven waarom we met dit onderzoek begonnen. Er wordt kort ingegaan op de resultaten van de onderzoekingen en met name op de overwegingen die leidden tot vervolgonderzoek.

Hoofdstuk II geeft een overzicht van de literatuur over dit onderwerp.

In de **Hoofdstukken III en IV** wordt het onderzoek beschreven naar de invloed op de KW van amoxicilline, cefaclor, cotrimoxazol, doxycycline, erythromycine, phenethicilline en roxithromycine. Deze studies tonen aan dat de KW wordt verstoord door amoxicilline en phenethicilline. De faecesconcentratie van Gram-negatieve bacillen, enterococcen of gisten nam niet toe na de andere middelen. We vonden echter vaker kolonisatie met lage concentraties van andere Gram-negatieve bacillen dan *Escherichia coli* (secundaire kolonisatie), vooral als de faecesconcentratie van *E. coli* was verlaagd. De concentratie van secundaire Gram-negatieve bacillen was dan echter niet hoger dan de concentratie van *E. coli* vóór behandeling. Na amoxicilline en phenethicilline was dat wel het geval. Het leek alsof een normale concentratie van *E. coli* nodig is om secundaire kolonisatie te voorkomen, en dat verlaging van de

concentratie van *E. coli* kolonisatie mogelijk maakt met andere Gram-negatieve bacillen in lage concentratie ("substitutie-kolonisatie"). We meenden daarom dat toename van secundaire kolonisatie in lage concentratie niet kon worden beschouwd als definitief bewijs voor verstoring van de anaërobe flora die de KW veroorzaakt.
In hoofdstuk IX toonden we echter aan dat eliminatie van *E. coli* met een lage dosis pefloxacin kolonisatie van de darm door een resistente challenge stam van *Klebsiella pneumoniae* niet vergemakkelijkte, terwijl deze stam de darm wel in hoge concentratie koloniseerde als clindamycine werd toegevoegd. Daarom zijn we nu van mening dat toename van secundaire kolonisatie een sterke aanwijzing vormt voor verstoring van de anaerobe flora die de KW veroorzaakt.

In **Hoofdstuk V** beschrijven we onderzoek naar de invloed van clindamycine, dicloxacilline, norfloxacin en minocyline op de faecesconcentratie van urobilinogeen. We waren tot de conclusie gekomen dat toedienen van resistente challenge-stammen nodig is om KW-verstoring aan te tonen door antibiotica waarvoor normaal voorkomende Gram-negatieve bacillen ongevoelig zijn. We schrokken daar echter voor terug en zochten naar een alternatief.
Er was aangetoond dat sterke verstoring van de KW kon worden aangetoond door toename van de faecesconcentratie van bèta-aspartylglycine, maar deze methode was niet gevoelig genoeg voor het aantonen van matige verstoring van de KW. Inmiddels was ook aangetoond dat urobilinogeen afwezig is in faeces van kiemvrije dieren en afwezig of sterk verlaagd is in faeces van dieren die waren behandeld met AMM die de KW sterk reduceren. Antibiotica waarvan werd verondersteld dat ze de KW niet verstoren hadden geen aantoonbare invloed op de faecesconcentratie van urobilinogeen. We wilden daarom weten of het mogelijk was de faecesconcentratie van urobilinogeen te gebruiken als een indicator voor de invloed van antimicrobiële middelen op de KW van mensen. Het bleek dat de faecesconcentratie van urobilinogeen sterk werd verlaagd door toediening van clindamycine en dicloxacilline. Deze middelen tasten ook de KW sterk aan. Na norfloxacin en na minocycline, waarvan werd aangenomen dat ze de KW niet verstoren, daalde de concentratie van urobilinogeen wel, maar niet statistisch significant.
We concludeerden dat afname van de faecesconcentratie van

urobilinogeen werd veroorzaakt door remming van de anaërobe flora van de darm, en mogelijk bruikbaar was voor onderzoek naar de invloed van AMM op de KW.
Later onderzoek toonde echter aan dat minocycline de KW verstoort in een deel van de vrijwilligers. Hetzelfde geld voor pefloxacin, dat vergelijkbaar is met norfloxacin. Het verschil in de resultaten met clindamycine en dicloxacilline enerzijds, en minocycline en norfloxacin anderzijds, werd dus veroorzaakt door een kwantitatief verschil in de aantasting van de KW-flora. De urobilinogeenmethode is dus niet gevoelig genoeg om een geringe aantasting van de KW aan te tonen.
Afname van de faecesconcentratie van urobilinogeen na toediening van AMM wordt veroorzaakt door remming van de metabole activiteit van de anaerobe flora die bilirubine-glucuroniden deconjugeert. Dit onderzoek wijst op de mogelijkheid dat remming van deze anaerobe flora de entero-hepatische kringloop kan onderbreken van stoffen die na excretie in de gal worden gedeconjugeerd door microbieel glucuronidase, bijvoorbeeld oestrogenen. Antibiotica die anaerobe flora aantasten kunnen dus mogelijk leiden tot falen van orale anticonceptie.

In de **Hoofdstukken VI, VII, VIII, IX en XI** wordt de invloed op de KW onderzocht van amoxicilline, cefotaxim, cephradine, clindamycine en pefloxacin.
De studies in de hoofdstukken III en IV waren uitgevoerd volgens de klassieke studie-opzet. Deze heeft echter een aantal ernstige tekortkomingen. We besloten daarom tot de volgende aanpassingen van de onderzoeksmethode.
1. Analyse van de resultaten in iedere vrijwilliger apart, in plaats van analyse van de resultaten vrijwilligers als groep. Uit de fluctuatie van de concentratie van Gram-negatieve bacillen in faeces van vrijwilligers berekenden wij dat een controleperiode en een behandelingsperiode van ieder tien dagen voldoende zijn om een tienvoudige verandering in de faecesconcentratie met statistische significantie te kunnen aantonen.
2. Toediening van challenge-stammen, resistent tegen de actieve faecesconcentratie in de darm. Op deze wijze zouden we niet langer afhankelijk zijn van toevallige aanwezigheid van resistente microorganismen.

3. Gebruik van selectieve voedingsbodems, teneinde de kans te verhogen op het aantonen van secundaire Gram-negatieve bacillen in aanwezigheid van een hoge concentratie van *E. coli*.

De nieuwe onderzoeksmethode werd in de genoemde hoofdstukken toegepast en besproken. We onderzochten de invloed van AMM op de faecesconcentratie van (1) Gram-negatieve bacillen als groep, van (2) secundaire Gram-negatieve bacillen die spontaan aanwezig waren, van (3) resistente Gram-negatieve bacillen die als challenge waren toegediend, van (4) Gram-positieve coccen en van (5) gisten.
Bij verstoring van de KW kunnen deze parameters voor de KW alle vijf positief worden. Bij verstoring van de KW bleek echter competitie van Gram-negatieve bacillen onderling voor te komen. De parameters 2 en 3 kunnen dus negatief blijven als de eigen *E. coli* ongevoelig is voor het toegepaste AMM en zelf snel genoeg de "ruimte" bezet, die is vrijgekomen door verstoring van de KW. Hieruit blijkt dat resistente challenge-stammen niet altijd de meest gevoelige indicator zijn voor verstoring van de KW. Dit is wel het geval als de challenge-stam geen competitie ondervindt van andere Gram-negatieve bacillen.
Bij aantasting van de KW en aanwezigheid van resistente stammen worden de parameters 1, 4 en 5 meestal alle drie positief. Dit blijkt echter niet altijd zo te zijn. Bij één van de vijf vrijwilligers steeg de concentratie van gisten niet na clindamycine (in tegenstelling tot de concentratie van Gram-negatieve bacillen en van enterococcen), en bij vier van de vijf vrijwilligers met verstoorde KW na cefotaxim (blijkens stijging van de concentratie van enterococcen en gisten) werd de concentratie van resistente Gram-negatieve bacillen niet hoger dan de concentratie van Gram-negatieve bacillen in de periode vóór behandeling. Dit wijst op de mogelijkheid van een zekere selectiviteit van verschillende soorten van de anaërobe KW-flora voor wat betreft hun remmende werking op groepen of soorten van de aërobe flora in de darm.

In tegenstelling tot onze verwachtingen bleek dat alle onderzochte middelen de KW verstoorden, hoewel niet in dezelfde mate en niet bij alle vrijwilligers. Uitgroei van Candida bleek een zeer betrouwbare indicator voor verstoring van de KW te zijn. Candida kwam bij de meeste vrijwilligers voor en is resistent tegen antibacteriële

middelen.
Aantonen van verstoring van de KW tegen Gram-negatieve bacillen door cefotaxim of pefloxacin is alleen mogelijk door resistente challenge-stammen toe te dienen. Hoewel we er niet in slaagden Gram-negatieve bacillen te verkrijgen die voldoende resistent zijn tegen de concentratie van pefloxacin in de darm tijdens behandeling met pefloxacin, konden we verstoring van de KW tegen Gram negatieve bacillen aantonen door toediening van de challenge stam na de pefloxacin-periode, namelijk bij twee van de drie vrijwilligers waarin verstoring van de KW tijdens behandeling bleek uit een significante stijging van gisten. De methode van challenge na behandeling is echter alleen mogelijk als verstoring van de KW langer duurt dan dat de actieve concentratie in de darm boven de MRC van de challenge stam ligt.

In **Hoofdstuk IX** onderzoeken we ook de bijdrage van Gram- negatieve bacillen in de darm, met name *E. coli*, aan de KW. We hadden aanwijzingen gevonden dat de actieve faeces-concentratie van quinolonen ongeveer gelijk is aan de diffundeerbare faeces-concentratie. De meeste Gram-negatieve bacillen hebben een MRC voor pefloxacin beneden 4 mg/l. We berekenden dat deze concentratie in faeces zou worden bereikt door orale toediening van 20 mg pefloxacin éénmaal daags. Deze dosis verwijderde Gram-negatieve bacillen uit faeces zonder een verandering te veroorzaken in de faeces-concentratie van enterococcen of gisten, en zonder kolonisatie van de darm door een resistente challenge-stam van *Klebsiella pneumoniae* te bevorderen. Dit toont aan dat Gram-negatieve bacillen in de darm geen bijdrage leveren aan de KW. Selectieve dekolonisatie van de darm van Gram-negatieve bacillen (dwz. dekolonisatie zonder de KW-flora aan te tasten) is dus mogelijk.

In **Hoofdstuk X** beschrijven we de invloed van parenterale toediening van pefloxacin op de faecesconcentratie van normaal aanwezige Gram-negatieve bacillen in de darm en van een zeer resistente challenge-stam. Gram-negatieve bacillen verdwenen snel uit de faeces, en de challenge stam slaagde er niet in de darm te koloniseren. Hoewel dit de meest resistente stam van Gram-negatieve bacillen was die we ooit in handen hebben kunnen krijgen, was toch de MRC van deze stam (56 mg/l) lager dan de diffundeerbare

faeces-concentratie van pefloxacin (160 mg/l). Met dit onderzoek kon dus niet worden bewezen dat dekolonisatie met 800 mg pefloxacin per dag selectief is. Het onderzoek was daar bovendien niet voor geschikt omdat het te kort werd voortgezet om individuele analyse van de gist-concentratie als parameter te kunnen gebruiken. Wel werd hiermee aangetoond dat parenterale toediening van pefloxacin zeer effectief is voor eliminatie van Gram-negatieve bacillen uit de faeces en voor preventie van nieuwe kolonisatie met zeer ongevoelige exogene bacteriën. Dit maakt het mogelijk pefloxacin parenteraal toe te dienen voor profylactische dekolonisatie van patiënten die niet oraal kunnen worden behandeld, bijvoorbeeld in verband met braken.

In **Hoofdstuk XII** worden de resultaten van onze onderzoekingen en de implicaties daarvan voor het antibiotica-beleid besproken.
Er wordt geconcludeerd dat deze onderzoekingen nieuwe argumenten leveren voor de veronderstelling dat de KW-flora uitsluitend bestaat uit anaerobe bacteriën. Het is daarom theoretisch mogelijk om infecties met aërobe micro-organismen te behandelen zonder risico op superinfecties. De meeste, zo niet alle, thans beschikbare antibiotica verstoren echter de KW-flora in tenminste een deel van de populatie. Effectieve profylaxe van infecties is niettemin reeds thans mogelijk, door regimes te ontwerpen die actieve faeces-concentraties bereiken hoger dan de MRC van normaal voorkomende aërobe bacteriën, vooral Gram-negatieve bacillen. Met deze middelen heeft men een lagere kans op het veroorzaken van bacteriële superinfecties dan met AMM die de KW verstoren en waarvan de faeces-concentratie lager is dan de MRC van veel aërobe bacteriën. In dit opzicht zijn clindamycine en penicillines de onveiligste, en quinolonen de veiligste thans beschikbare AMM. Het zoeken naar middelen die de KW niet aantasten dient echter te worden voortgezet.

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Dr. H.J.A. Wijnne, statisticus. Beste Herman, jouw kennis van de "single patient" statistiek was essentieel voor dit onderzoek. Zonder jouw bijdrage was het onmogelijk geweest de onderlinge beïnvloeding van diverse groepen micro-organismen te bestuderen.
De ruim 40 vrijwilligers die aan de onderzoekingen deelnamen vervulden een heldenrol. Weinig personen zijn bereid een maand lang dagelijks faecesmonsters in te leveren en het is opvallend hoe griezelig veel gezonde personen (inclusief artsen) het vinden om antibiotica te slikken, laat staan om tien dagen met een naald in de arm rond te lopen om zich dagelijks intraveneuze injecties te laten toedienen. Nog moeilijker wordt het als bovendien resistente challenge-stammen moeten worden geslikt. Het algemene gevoel daarover wordt waarschijnlijk weergegeven door een referent van de Journal of Antimicrobial Chemotherapy, die schreef "I don not find

a real ethical problem, although I find the insouciance about the possible virulence of the Klebsiella challenge impressive".
Ik prijs mij zeer gelukkig dat er in mijn omgeving personen waren te vinden die zich van dit alles niets aantrokken en gewoon meededen.

CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 3 oktober 1944 te Amsterdam. In 1962 behaalde hij het diploma Gymnasium bèta aan het Katholiek Gelders Lyceum te Arnhem. Daarna studeerde hij farmacie aan de Rijksuniversiteit te Utrecht. Daar behaalde hij het doctoraal examen in 1969 en het apothekersexamen in 1970. Hij volgde de opleiding tot ziekenhuisapotheker in de apotheek der R.K. Ziekenhuizen in Eindhoven (opleider dr. F.C. Bedaux) en werd in 1973 als zodanig geregistreerd.
Vanaf 1 juli 1973 is hij als ziekenhuisapotheker verbonden aan het Canisius-Wilhelmina Ziekenhuis. Tevens is hij toezichthoudend apotheker van de verpleeghuizen St. Joachim en Anna te Nijmegen, Waelwick te Ewijk en Margriet te Nijmegen en van het Psychiatrisch Centrum Nijmegen.